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ARNE MÜNTZING
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Institute of Genetics,
LUND, SWEDEN

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ENDO-DUPLICATIONAL MEIOSIS IN ALLIUM ODORUM

By *ARTUR HÅKANSSON* and *ALBERT LEVAN*
INSTITUTE OF GENETICS, LUND, SWEDEN
(Received September 15th, 1956)

I. INTRODUCTION. METHODS

ALLIUM odorum, a species with wide distribution in temperate Asia and often grown in botanic gardens, has been repeatedly investigated embryologically because of its antipodal embryos. Early investigators, SCHÜRHOFF and HABERLANDT, seem to have studied a diploid *odorum*-form ($2n=16$), while most later workers have found their *odorum*-plants to be tetraploid ($2n=32$). The present authors have grown forms from some twenty botanic gardens, all these *odorum*-forms being tetraploid. The study of herbarium specimens shows, however, that *Allium odorum* includes morphologically widely diverse types, of which certainly only a few are at present available in botanic gardens. KURITA (1953) concludes from the somatic chromosome morphology and from the meiotic pairing that *Allium odorum* is an autotetraploid.

Embryologic investigation into tetraploid forms (see MODILEWSKY, 1931, HÅKANSSON, 1951) have shown the embryos to be formed by parthenogenesis, while undoubtedly fertilization of the polar nuclei is necessary, no endosperm developing in emasculated flowers. The antipodals usually have the appearance of a second egg apparatus, antipodal eggs also developing parthenogenetically. The zygoid chromosome number is the rule in the embryo sacs, although, exceptionally, embryo sacs with reduced chromosome number are found. MODILEWSKY (1931) describes meiosis in pmc:s and emc:s. In the latter case he finds, on the one hand, a normal meiosis leading up to »16 Gemini» and, on the other hand, a deviating meiosis type leading to the double number of chromosomes. Although he refers to the process involved in this chromosome doubling with the misleading term »Restitution» and the nucleus resulting, »Restitutionskern», it is quite clear from his descriptions that he does not mean an ordinary restitution mechanism. He is speaking of »eine Restitution in dem praediakinetischen Stadium» (p. 36), and in his Fig. 35 a first metaphase is represented with about 32 elements,

»mittlerer Länge und Dicke im Vergleiche mit den heterotypischen und somatischen Chromosomen» (p. 45).

The present paper focuses on the chromosome behaviour during female meiosis. Male meiosis will be analysed as a background to this. The sectioned material, mainly used for this study, was supplemented with squashed material, favourable for a critical analysis of the meiotic details in pmc:s and emc:s. Separate anthers or ovules were fixed for 24 hours in equal parts of Carnoy 3 : 1 and 60 % acetic acid, hydrolyzed for 7 minutes, and stained two hours in Feulgen. The pmc:s were squeezed out of the anthers, the remnants of the anthers being removed before squashing. Single ovules were placed on a non-siliconed coverslip and squashed against a siliconed slide. In both cases permanenting was immediately made according to the dry-ice-euparal method. The pollen mitosis was fixed in Bouin-Allen and stained in gentian-violet according to LEVAN (1932).

II. EMBRYOLOGICAL OUTLINE

Since the present observations of the general embryologic course do not differ essentially from those of MODILEWSKY (1925), only a few brief data will be communicated. As in other *Allium* species the ovule has a rather small nucellus, surrounded by two integuments. The nucellus contains one, or very exceptionally two, emc:s without any cover cell. Volume growth associated with endomitosis has been observed in solitary nucellus cells although much more seldom than in *Allium nutans* (HÅKANSSON, 1951). The es is bisporous, its development following the *Allium*-type. During its growth the lateral and apical nucellus cells are destroyed, the organized es thus being surrounded by integument tissue.

The general outline of the meiosis in the emc is represented in Fig. 1. Fig. 1 *a* shows an emc at pachytene, *b* at first metaphase. The direction of the spindle at this first metaphase is characteristic: its axis is often transverse or oblique in relation to the length axis of the emc. Thus the metaphase plate is seen in polar view in Fig. 1 *b*, which is a longitudinal section. Consequently the anaphase I groups may be lying beside each other as in Fig. 1 *c*, forming two large interkinetic nuclei on about the same level in the cell (Fig. 1 *e*). More often, however, the dyad nuclei are lying at somewhat different levels, an oblique cell wall being formed between them (Fig. 1 *d*). Possibly the unusual direction of the first spindle is connected with the considerable width of the emc.

In exceptional cases both dyad nuclei may divide, forming binucleate

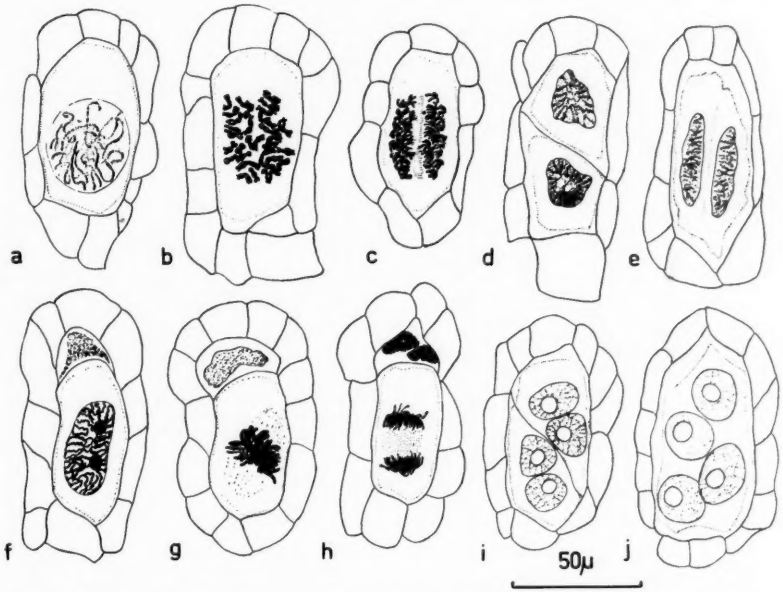

Fig. 1. Outline of female meiosis. *a*: pachytene, *b*: metaphase I, *c*: anaphase I, *d*, *e*: interkinesis, *f*: prophase II, *g*: metaphase II, *h*: late anaphase II, *i*, *j*: deviating stages, *i*: binucleate dyad cells, *j*: monad with four nuclei. — ×400.

dyad cells (Fig. 1 *i*). Since the occurrence of two es:s in the same nucellus is very rare, evidently one of the dyad cells in such ovules degenerates. Failure of cell formation after the first divisions rarely leads to four nuclei in a monad cell (Fig. 1 *j*). Later on, small es:s lacking vacuoles may be seen, which have probably originated from the division of the nuclei in such cells.

However, in the normal case the emc is divided by a transversal wall into one upper small cell, the further development of which fails, and one lower cell, which forms the es. After the first meiotic division an unusually long time interval elapses before the second division. The nucleus of the dyad cell has thus not the ordinary interkinetic structure but rather the appearance of a deep resting stage. Such a delay in the division of the dyad nucleus is known in connection with the formation of non-haploid es:s, for instance, in a dyad-forming mutant of *Datura* (SATINA and BLAKESLEE, 1935) or more exceptionally in apomictic *Archieracium* (BERGMAN, 1941). The appearance of the second

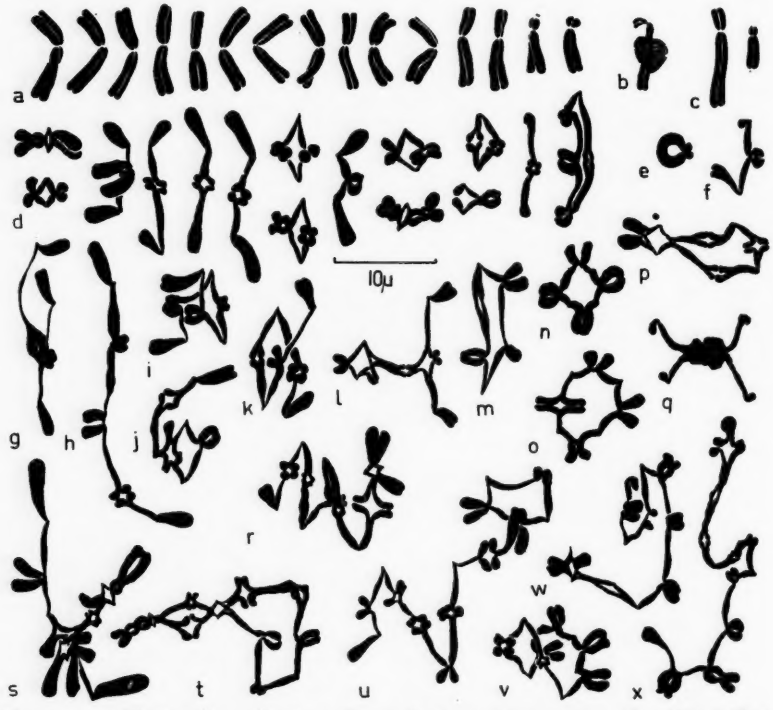

Fig. 2. *a*—*c*. First pollen mitosis. *a*: the metaphase chromosomes of a first pollen mitosis, *b*: the nucleolar chromosome at prophase, *c*: deviating chromosome types in the pollen, *d*—*x*: first division of the male meiosis, *d*: the chromosomes of one pmc, *e*: ring univalent, *f*: heteromorphous bivalent, *g*: trivalent, *h*—*q*: quadrivalents, *r*—*x*: higher complexes. — ×1500.

meiotic division is seen from Fig. 1 *f*—*h*. After this division a vacuole appears between the daughter nuclei, and the stage of binucleate es is attained.

III. THE IDIOGRAM OF ALLIUM ODORUM

The *odorum*-types studied by us have the same chromosome morphology as described by KURITA (1953). Fig. 2 *a* gives the chromosomes of the first pollen mitosis, which stage yields a somewhat better picture of the chromosome details than root mitoses, because of the lower chromosome number and the more pronounced contraction of the chromo-

somes. All the chromosomes are metacentric. Six chromosomes of the basic 8-set are more or less submedially attached, ranging in size between 8 and 11 μ. These chromosomes are not individually recognizable. The two remaining chromosomes of the basic set are more asymmetric. The largest one of these is about 9 μ in length, its two arms approaching the length relation 2 : 1. The smaller one, with its length of 6 μ, being the smallest member of the idiogram, has an arm index of 5 : 1. This chromosome, having a small satellite on its shorter arm, is always readily identifiable. The nucleolus is formed at the satellite of this chromosome (Fig. 2 *b*). It corresponds to the so-called s_1 chromosome, present in the idiograms of most *Allium*-species.

Clearly deviating chromosome types were sometimes observed in the pollen cells. Two such instances are pictured in Fig. 2 *c*. Their origin is natural considering the abnormal types of pairing observed during meiosis (see below).

The chromosome number of the pollen grains varied as a consequence of the multivalent formation at meiosis. In 100 cells the following numbers were recorded:

Chromosome number	13	14	15	16	17	18	Mean
Number of cells	2	10	28	40	15	5	15,7

Thus, a small chromosome elimination has shifted the distribution somewhat to the left. This is an observation, common among autotetraploid *Allium* species (*e. g.* LEVAN, 1933, Table 2, p. 110). The pollen mitosis analysed in Fig. 2 *a* had only 15 chromosomes, one of the long submedian chromosomes being absent.

IV. MALE MEIOSIS

As observed by KURITA (1953) male meiosis of *Allium odorum* is characterized by the frequent occurrence of quadrivalents. The chromosomes of one squashed pmc are drawn *in situ* in Fig. 3 *a*. They comprise three quadrivalents (IV), two trivalents (III), six bivalents (II) and two univalents (I). Another cell, analysed in Fig. 2 *d*, had only one IV and 14 II:s. In one slide twenty-five cells were analysed as to their chromosome associations (Table 1). It was found that six of these contained higher associations than IV:s, in one case as many as twelve chromosomes being joined by chiasmata. No cell among these twenty-five had exclusively II:s. The frequency per pmc of II:s and IV:s, respectively, varied as follows:

TABLE 1. *Chromosome associations in twenty-five cells at first metaphase of male meiosis.*

Associations																					
	XII	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	VIII	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	VII	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	VI	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	V	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	IV	1	—	4	3	5	3	7	6	5	5	4	4	3	3	3	3	2	2	1	
	III	1	1	—	—	—	—	—	—	—	—	1	—	2	1	—	—	1	—	—	
	II	—	6	4	6	3	7	2	4	6	5	6	7	6	8	10	8	10	12	4	
	I	1	2	—	—	—	1	—	—	—	2	1	2	2	1	—	4	1	—	—	
Number of cells		1	1	1	1	1	1	1	1	2	1	2	2	1	3	2	1	1	1	1	

Total of cells analyzed: 25

	0	1	2	3	4	5	6	7	8	10	12	14	Mean
II	1	—	1	1	2	1	7	3	4	3	1	1	6,76
IV	1	2	2	9	5	4	1	1	—	—	—	—	3,44

Thus, six to seven II:s and three to four IV:s was the typical case in the material analysed. It should be observed that the selection of pmc:s for analysis is necessarily somewhat biased in favour of analysable, thus less complex, associations. The real situation should probably be a shift towards the more complicated configurations, that is towards the left, in Table 1.

The appearance of the various meiotic configurations is seen in Fig. 2. The bivalents vary from very simple rod bivalents with only one chiasma to highly complex ones with a row of chiasmata all along their length. The chiasma situation, too, is probably in reality more complex than indicated by the pictured cases. For instance, in Fig. 2 *d* the chiasma frequency of the individual II:s was

Number of chiasmata	1	2	3	7
» » II:s	5	6	2	1

and that of the single IV 6, that is 2,2 chiasmata per II. The cell of Fig. 3 *a* has similar chiasma numbers. The average number of chiasmata per cell is probably somewhat higher, the cells reproduced being selected for their clear chiasma conditions.

The degree of terminalisation of the chiasmata is low. A clear tendency to localization of chiasmata in the neighbourhood of the centromeres is apparent. It often happens that one or two bivalents in a cell have a high number of chiasmata. In such bivalents the usual ring-shape is modified to a somewhat irregular »sausage»-shape, the centromeric opening being little larger than the other loops in between the chiasmata. Such a bivalent form is the prevailing type in many liliaceous genera, for instance in *Hyacinthus* and *Lilium*. It should be observed that a great variation in chiasma number and bivalent form within the cell has been observed, especially in genetically imbalanced types, as for instance, in F_2 plants of the species cross *Allium Cepa*× *fistulosum*, of which the one parental species had normal random chiasmata and the other had strict chiasma localization (LEVAN, 1941, Fig. 4, p. 265).

Univalents are frequent. They are of normal shape, resembling somewhat over-contracted somatic chromosomes. Their centromeric constrictions are usually visible. Quite often one univalent was seen to have its two arms joined together by a chiasma: »ring-I» (Fig. 2 *e*). Some-

times clearly non-homologous chromosomes were associated into a bivalent (Fig. 2 *f*).

The tendency towards the development of a high number of chiasmata, seen in the II:s, and the tendency to chiasma localization near the centromeres are again met with in the multivalents. This condition often makes them very hard to analyse. The IV:s include simple chains (Fig. 2 *h*, *i*, *k*), rings (Fig. 2 *m*, *n*, *o*), frying pans (Fig. 2 *j*), but also more complex types. Triple and quadruple chiasmata were seen in III:s and IV:s (Fig. 2 *g*, *q*). Usually higher complexes could be completely analysed only when they constituted simple chains or rings. Some of these types are pictured in Fig. 2 *r*—*x*, including VII:s, VIII:s, and X:s. In many of them the tendency to chiasma localization is quite evident (Fig. 2 *r*, *s*, *t*, *w*).

First anaphase is characterized by a frequent occurrence of unclean separation. The most common irregularity was the lagging of univalents. Among 80 pmc:s recorded, 29 had clean separation, 42 had one to four laggards, usually entire univalents, but also (in 14 cases) single chromatids, and (in five cases) both univalents and single chromatids. In nine cases single chromatid bridges joined univalents from pole to pole: in five of these no free fragment was seen, and in four cases a fragment of varying size was present. One of the former bridges, and the four latter ones are drawn in Fig. 3 *b*. The comparatively common occurrence of single chromatid bridges with fragments (5 %) is evidence of inversions in the chromosomes of *Allium odorum*.

Briefly, it may be stated that the normal male meiosis of *Allium odorum* indicates an autotetraploid origin of this species. The presence of intrachromosomal chiasmata in univalents, of heteromorphous bivalents, of higher chromosome associations than IV:s, and of bridge formations at anaphase indicate that numerous structural changes have been superimposed on the original autogenomatic situation. In addition, the considerable variability in chiasma number and localization even within the same cell points to some failure of balance within the meiotic variables. All these conditions are compatible with the comparative insignificance of the pollen in an apomictic though pseudogamous species.

Among eleven slides scrutinized, representing the anthers of some six flowers, all of which were at or about the first metaphase, four cells were found with a deviating meiosis. Since this deviation was strikingly similar to the meiosis typical of the emc:s, they will be described here, although the detailed data on this type of meiosis are reserved for the next section.

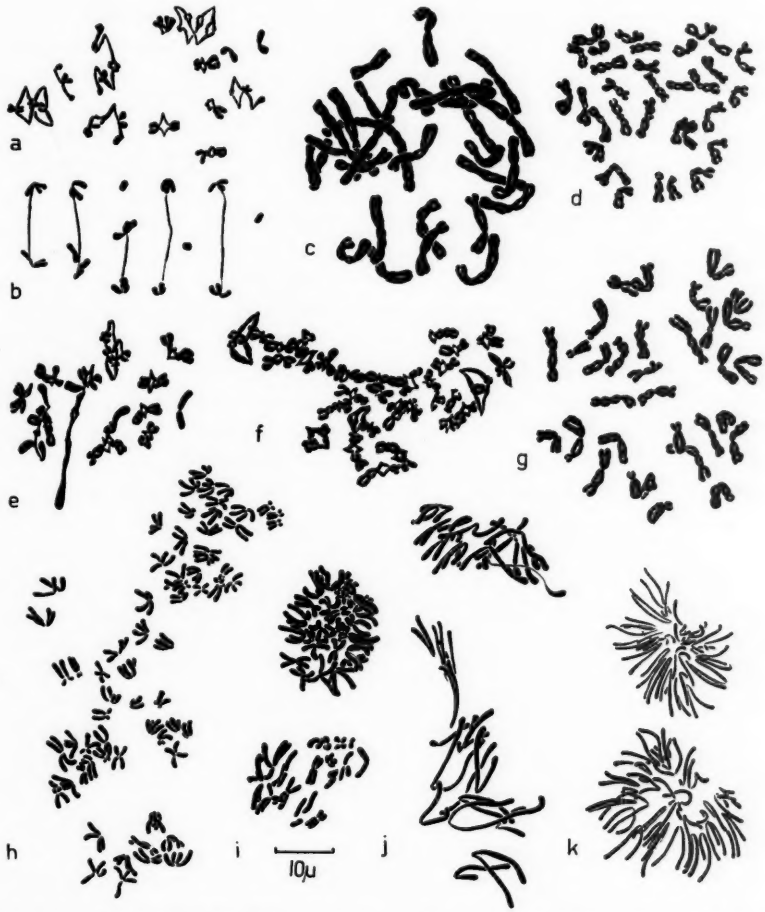

Fig. 3. *a—d*. First meiotic division on the male side. *a*: the chromosomes of one metaphase I cell, *b*: instances of bridge formations at anaphase I, *c*, *d*: deviating meiosis, *c*: at diakinesis, *d*: at metaphase I, *e—h*: first meiotic division on the female side, *e*: metaphase I cell with the zygoid number of chromosomes, *f*, *g*: metaphase I cells with the double zygoid number of chromosomes, *f*: deviating type, *g*: the type normal for the female side, *h*: anaphase I, *i*: second metaphase, two sections, *j*: second anaphase, azygoid number, *k*: second anaphase, zygoid number. — ×900.

Two of these cells are pictured in Fig. 3 *c* and *d*. They were pmc:s of ordinary size, at diakinesis (*c*) and metaphase I (*d*), respectively. Each of them contains 32 elements of the size of ordinary bivalents.

Some of these elements were clearly seen to be quadripartite and to contain chiasmata. Without any doubt each element corresponded structurally and mechanically to a bivalent. The number of chiasmata was very high, each single bivalent having as many chiasmata as found only in few bivalents at normal meiosis. Thus, the cell of Fig. 3 *d* had five chiasmata per II as the commonest case (variation 4—7, mean 4,75 chiasmata per II).

The shape of the bivalents corresponds completely to the »sausage-shape», observed in the normal type of meiosis in those bivalents having the highest number of chiasmata. The centromeres are located in a very small loop, closely limited by one chiasma on each side. This condition makes the centromeric region more flexible than the rest of the bivalent, causing the bivalents to assume a V-shape on the plate with the apex of the V turned inwards, just as is typical of somatic chromosomes. The significance of this bivalent type will be evident after the description of the female meiosis.

V. FEMALE MEIOSIS

The most common meiosis type on the female side agrees with the one just described for the male side as deviating meiosis. The significant stage is the first meiotic metaphase, when 32 bivalents are formed of a »sausage-type», strikingly different from the ordinary bivalent type of the genus *Allium*. The prophase stages leading up to this situation are all highly ordinary. At the earliest moment when the chromosomes are visible, they have the appearance of a typical pachytene stage (Fig. 1 a). Very often two threads may be discerned at this stage. Gradually they fall apart, becoming wound around each other in a spiral (Fig. 4 *a*). Thereafter the normal contraction takes place, and the diplotene and diakinesis bivalents are developed (Fig. 4 *b*—*i*). These stages are difficult to analyse, the fixation often being unfavourable. Chiasmata are seen, however, and the centromeric regions are very often held closely together.

Metaphase I (Fig. 1 *b*, 3 *g*, 4 *j*—*l*, *s*) is a very favourable stage for the study of the bivalents both in sectioned and squashed material. It is now evident that each bivalent consists of four chromatids and that numerous chiasmata are present. A superficial similarity to somatic chromosomes is caused by the very close connection of the four chromatids at the centromeres. Sometimes the impression of somatic diplochromosomes is very strong (Fig. 4 *j*, *k*), and it may well be that the centro-

meres do not separate until middle metaphase, so that the bivalents really are diplochromosomes all through the prophase stages. At metaphase, however, the bivalents do not behave as diplochromosomes. As is well known (*e. g.* LEVAN and HAUSCHKA, 1953) each chromosome, arising when the centromere of a diplochromosome divides, behaves as a mitotic chromosome. that is the centromeres auto-orientate. But in *Allium odorum* the two members of the diplochromosomes co-orientate as soon as the centromeres have divided.

A typical metaphase plate (the one in Fig. 1 *b*) is analysed in Fig. 4 *p*. The bivalents are strikingly uniform, in almost all of them there is one chiasma on each side of the centromere, the centromeric loop often being smaller than the lateral loops. In one bivalent the two centromeric chiasmata are the only ones present, the bivalent taking the cruciform shape typical of *Allium* species with chiasma localization, as *fistulosum* and *porrum*. Usually one or more chiasmata are present besides the two centromeric ones. In this cell the chiasma number per II varied from two to six with a mean of 4,06.

Evidently each meiotic bivalent in this female meiosis, as well as in the deviating male meiosis, corresponds to one univalent chromosome of the *odorum* idiogram. An endomitotic reproduction of the chromosome thread has made it possible for each univalent to take the shape of a bivalent and to function as a bivalent all through meiosis. Because each univalent is forming a bivalent by itself without interference of any homologous pairing, we propose the term *autobivalent* for this structure.

The autobivalents are of interest in comparison with the normal bivalents, as developed in the pmc:s. The autobivalent should represent the limit type of bivalents originating in this species when the conditions of pairing are as favourable as possible. In the autobivalents the two mating threads have existed alongside each other from their beginning. No time has been needed for looking up the mating partner. The evident tendency to localization of the chiasmata towards the centromeric regions should be seen in connection with the fact that the centromeric regions are probably the latest ones to separate at an endomitosis. On the other hand a certain tendency to centromeric chiasmata was seen also on the male side. Quite likely the *Allium odorum* has a genetic tendency for starting the pairing and the chiasma formation at the centromeric regions, proceeding from here towards both ends.

A variant of this uniform and very characteristic metaphase I, typical of female *Allium odorum*, is the type represented in Fig. 3 *f*, analysed in Fig. 4 *q*. It agrees with the typical case in its main features: there are 32

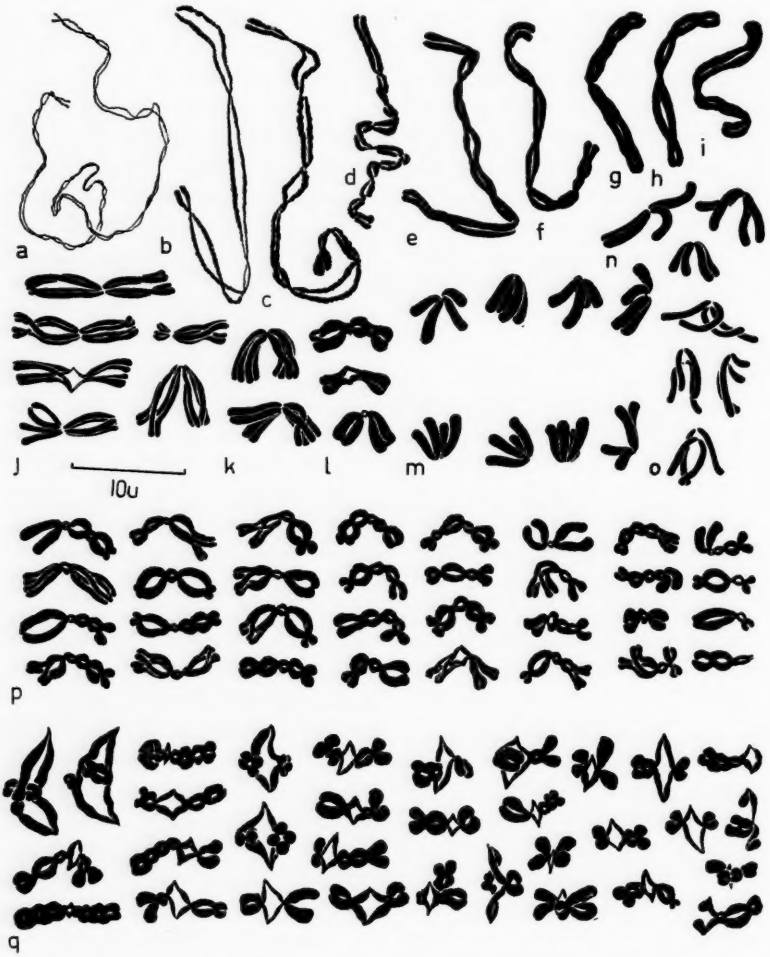

Fig. 4. Meiosis on the female side. *a*—*d*: diplotene, *e*—*i*: diakinesis, *j*—*l*: metaphase I, *m*: anaphase I, *n*: metaphase II, *o*: beginning anaphase II, *p*: analysis of the metaphase I plate of Fig. 1 *b*, *q*: ditto of Fig. 3 *f*. — ×1700.

bivalents, and the chiasma frequency is about the same (variation from two to nine chiasmata per II, mean 4,4). The main difference is the greater variation in bivalent type together with a less strict localization of the chiasmata. Typical *Allium* ring bivalents occur with the centro-

meric loop predominating, and even rod bivalents are found (*e. g.* one of the s_1 bivalents to the extreme right of the Fig.). This meiosis type is very rare, as a matter of fact only this one instance of it was seen among the some hundred metaphases observed. Its deviating behaviour may be interpreted by the assumption that the centromeric regions fall apart earlier than usual after the endomitosis, so that chiasma formation starts at some distance from the centromeres. This type is similar to the one most common in the other apomictic *Allium*, *viz. Allium nutans*. The main difference between the female meiosis of these two species may be referred to a different timing of the elementary process of the endomitosis.

The first anaphase (Fig. 3 *h*) has been observed many times. It is perfectly normal, the separating chromosomes having the four arms typical of metacentric anaphase I chromosomes (Fig. 4 *m*). The number 32 at each pole has been counted in many cases. As mentioned above, the resulting interkinesis enters a deep resting stage with complete disorganization of the chromosomes. The second prophase (Fig. 1 *f*) leads over to a metaphase, which has only been observed a few times. Here the chromosomes have the typical cross-shape (Fig. 4 *n*) with no relational spiral between the chromatids. Fig. 4 *o* shows early anaphase II, and Fig. 3 *k* a later anaphase II. The chromosomes of this stage are long and thin, and often very faintly stained. They are considerably larger than mitotic chromosomes in the surrounding nucellar tissue.

It occurs exceptionally that the meiosis on the female side takes the course of an ordinary male meiosis, forming es:s with reduced chromosome number. This variant may be recognized already at pachytene, the nuclei being smaller than the normal ones and containing paired pachytene threads, now and again exchanging univalent threads with each other, as is typical of multivalent formation. At metaphase I similar pictures were seen as described above for the pmc:s. Fig. 3 *e* is such a case, in which one I, II:s and multivalents are seen. Its chromosome number is clearly not doubled.

A few such cases were observed, several of them in the same individual plant, which may indicate a genotypic tendency of this plant to this type of meiosis. It would be of interest to ascertain whether certain environmental conditions favour one or the other of the meiotic types. Such experiments are planned.

This meiosis leads to es:s with only 16 chromosomes. An instance of an anaphase II with 16 chromosomes is pictured in Fig. 3 *j*.

VI. DISCUSSION

1. The autobivalent formation of Allium odorum

The female meiosis of *Allium odorum* differs from the male meiosis in the following respects: (1) Only bivalents are formed, the male meiosis showing a high frequency of multivalents; (2) the bivalents are present in the zygoid number; (3) the bivalents are characterized by a strikingly uniform shape, and their chiasma frequency is high.

It should be mentioned here, in passing, that the other *Allium* apomict studied by us, *Allium nutans*, has the same type of female meiosis. It should be observed, however, in relation to point (3) that the bivalents of female *nutans* are more variable in type than in *odorum*, the typical ring-shape of *Allium* bivalents predominating. A striking deviation found in the female *nutans* meiosis is the rather frequent occurrence of two endo-duplications in meiotic prophase, leading to diplo-bivalents with eight chromatids. So, even though differences occur between the two species, there is no doubt that, generally speaking, they belong to the same type of meiotic modification, both being cases of endo-duplicational meiosis (HÅKANSSON, 1951, HÅKANSSON and LEVAN, unpublished).

The entire body of existing evidence suggests the following explanation for the data observed: each premeiotic chromosome has undergone one extra division during meiotic prophase, allowing each univalent to develop into a bivalent, here called *autobivalent*. Before the zygotene stage each univalent is subjected to an endo-duplication, which will result in the mating tendency, normally acting between the homologues, being already satisfied. Thus, the pairing takes place between the two sister halves of each univalent chromosome. This is a mode of pairing which would reasonably be expected to predispose to a high degree of chiasma formation. The longer the centromeres hang together after the endomitosis, the better the opportunity for chiasmata to be formed in the centromeric region. As already suggested above, variations in the time of separation of the centromeres after the endo-duplication may cause variations in the chiasma distribution: a late separation will favour chiasmata close to the centromere, resulting in bivalents of the *odorum* type, while an earlier separation will interfere with the chiasma formation in this region, giving ring or rod bivalents as in *nutans*.

It is impossible to decide the exact moment of the extra chromosome reproduction. The exclusive formation of bivalents indicates, however, that the products of endomitosis cannot have fallen apart before the onset of zygotene, otherwise occasional multivalents would have been

expected. The time limit backwards for the endomitosis is more difficult to determine. It may even be theoretically possible that the extra chromosome division appearing in the emc may be that of the last archesporial mitosis, which has reverted before its completion. In our opinion, however, an endo-duplication in the young emc nucleus is more probable. We base this opinion on the fact that we have never seen any clear evidence of restitution mitosis in the subepidermal layer of very young ovules, although the early stages have been carefully searched for such mechanisms. Also, the regular shape of very young emc nuclei, showing no evidence of having gone through a restitution, points in the same direction. In addition, endomitotic processes are very common in nucellus cells, especially in *Allium nutans*. Mitoses with diplochromosomes and quadruplochromosomes were repeatedly seen. This feature is found also on the male side of *Allium nutans*, pmc:s with doubled chromosome number being frequent.

The formation of autobivalents, although typical in every mechanical respect, has genetically no meiotic significance. The failure of recombination and the restoration of the zygoid chromosome number make the present mode of propagation equal to clonal propagation. This is a mechanism that will accumulate heterozygosity. Structural heterozygosity was demonstrated in male meiosis of *Allium odorum*. In the present type the male meiosis constitutes a side branch of the germline with no evolutionary significance, as long as it only functions for the endosperm development.

This genetical situation is somewhat different, if the premeiotic doubling occurs early enough to permit the halfchromosomes to separate from each other before zygotene, as seems to be the case in some of the parallel cases discussed below under 3. In those cases there may be an opportunity for occasional homologous pairing with subsequent segregation.

2. The meiosis of Allium odorum, as related to meiotic mechanisms in other apomictic organisms

No doubt *Allium odorum* is a true apomict. The occasional formation of es:s with azygoid chromosome number is known in many apomicts, for instance, quite often in a Swiss *Poa alpina* apomict (the St. Gotthard form, HÅKANSSON, 1944). Even in *Archieracium*, which has always been regarded as an obligate apomict, »one cannot completely reject the idea of the existence of ± reduced embryo sacs» (BERGMAN, 1941, p. 34).

Poa alpina is partly sexual, which may be true of *Allium odorum*, too, though this needs confirmation.

Apomictic seed formation (agamospermy) in angiosperms may be combined into two main types: (1) the embryo is formed adventitiously from a cell outside the es, this case involving the formation of a normal reduced es, the egg of which generally degenerates, and (2) the embryo is formed through parthenogenesis in an es with the zygoid chromosome number. In the latter case the es may be formed from the emc (diplospory), or from another cell of the ovule (apospory). The endosperm is formed either by autonomous division of the polar nuclei, or after their fertilization (pseudogamy).

The formation of a diplosporous es will necessitate certain modifications of the meiosis (*cf.* GUSTAFSSON, 1946). These changes range from a meiosis with more or less complete asyndesis to ordinary mitosis. The following three types have all in common the non-formation of bivalents: (1) formation of a restitution nucleus in the first division, the second division having the zygoid number of chromosomes; (2) meiosis is replaced by an ordinary mitosis; and (3) the so-called pseudo-homeotypic division, in which univalents form a metaphase plate, divide, and give dyad nuclei with zygoid number. The third type has been contested with the argumentation that a transient stage of restitution may have been overlooked (FAGERLIND, 1947, BATTAGLIA, 1948, 1951). Our *Allium* cases fall outside this grouping, involving as they do a typical meiotic process. The only similar case among plants is found in ferns, as will be discussed later.

In the animal kingdom apomixis has a somewhat different spectrum of types, many of them involving true meiotic processes. WHITE (1954) distinguishes between (1) ameiotic and (2) meiotic parthenogenesis. These groups correspond roughly to SUOMALAINEN's (1950) apomictic and automictic types, respectively. It should be observed, however, that meiotic parthenogenesis includes SUOMALAINEN's automictic type with the azygoid number of bivalents, as well as types with the zygoid number of bivalents, discovered later.

The common denominator in all apomictic types of reproduction is the necessity of maintaining the chromosome number constancy. This is accomplished in the simplest way just by changing meiosis in the direction of mitosis, that is by cancelling the chiasma formation in meiotic prophase. The zygoid number of univalents is transferred, directly or *via* a restitution nucleus to the es or the egg. This method

of avoiding the chromosome number reduction is the predominating mechanism in plants and animals.

In meiotic parthenogenesis more subtle mechanisms have to be resorted to. Some of these will be briefly considered. A premeiotic doubling will enable a normal meiosis to take place, restituting the zygoid chromosome number. This is the mechanism of our *Allium* forms and will be considered in greater detail below.

More often meiosis starts out with normal chromosome number, and the reduction following is compensated for by automixis, this mechanism being common in animals. Automixis is a union of nuclei; usually the egg nucleus and the polar nucleus of the second division fuse; rarely there is fusion between cleavage nuclei (*Solenobia triquetrella*, SEILER, 1939). However, cases are known, which might be interpreted as abbreviated automixis (*Solenobia*, and other apomicts). The second anaphase may not be completed, a restitution nucleus being formed; or the second division may be totally omitted, the chromosome halves, which should normally separate at second anaphase, falling apart inside the nucleus. Mechanisms involving the omission of the second divisions are known from other animals as, for instance, a diploid parthenogenetic form of *Artemia salina* (BARIGOZZI, 1944).

An apomixis resembling the automictic parthenogenesis seems hardly to occur in higher plants. A *Datura* mutant, appearing after X ray treatment and regularly forming zygoid bisporous es:s, does not show parthenogenesis (SATINA and BLAKESLEE, 1935). Omission of the second division was also observed exceptionally in apomictic *Archieracium* (BERGMAN, 1941). Tetraploid es:s, originating by »double non-reduction», and more rarely diploid es:s have been described in a hybrid of *Musa* (DODDS and PITTENDRIGH, 1946); the exact mode of doubling, however, is not known in this case. BATTAGLIA (1951) describes the occasional formation of tetraploid es:s in *Rudbeckia laciniata* through »a birestitution nucleus», developed by »the formation of a restitution nucleus at the end of each of the two pseudomeiotic divisions which replace the first and second meiotic divisions» (*l. c.*, p. 721); this development may be present in the non-apomictic *Leontodon hispidus*, too.

A remarkable mechanism was discovered in certain species hybrids of *Saccharum* (BREMER, 1949). Here a doubling by endomitosis takes place with great regularity in the innermost of the four megaspores. Sometimes an endomitosis may occur already with one of the dyad cells, in which case the es will become tetraploid. This mechanism deviates

from the previous ones by involving one or two additional chromosome reproductions.

The omission of the second meiotic division on the male side has been observed in several *Allium* species, in some cases with a frequency reaching very nearly 100 % of the pollen (LEVAN, 1931). This process, leading to the formation of dyad pollen with zygoid chromosome number, was called monokinetic meiosis (LEVAN, 1936). In a *Musa* hybrid (DODDS and SIMMONDS, 1946) either the second, or both divisions, may be cancelled, resulting in pollen dyads with zygoid, or monads with double zygoid chromosome number. Similar end results have been achieved by temperature treatment (MATSUURA, 1937, BARBER, 1940) or colchicine treatment (LEVAN, 1939).

In pmc:s of *Hieracium robustum* (GENTCHEFF and GUSTAFSSON, 1940) an endomitotic process during meiotic prophase may lead to the double zygoid number of univalents at metaphase. This mechanism, called »double reproduction», is essentially similar to that of the female *Allium odorum*, although no bivalents were formed, and the mechanism of course is without significance for the germline of the species.

It may be disputed whether the monokinetic meiosis should be enrolled among the endomitotic doublings. Since the doubling inside the dyad nuclei is already completed, except for the separation of the centromeres during the preceding (meiotic) prophase, it is different from ordinary endomitosis. On the other hand, the final doubling of the chromosome number takes place inside the nuclear membrane. Maybe, this kind of doubling should be a subtype of endomitosis, called »endohomeotypic division».

3. Some cases comparable to the Allium case

As shown above, the apomictic *Allium odorum* and *nutans* have developed on the female side a meiotic mechanism for counteracting the lack of fertilization, unparalleled in Phanerogams. It is interesting to find that the similar existing cases are spread out among such diverse groups as ferns, worms, and cockroaches, a good illustration of the potential similarity of chromosomes, wherever found.

Although somewhat differing in detail, these cases have in common the general mechanism of a chromosome number doubling before the meiotic division. The doubling may be effected in various ways: (1) a real restitution mitosis is seen in the archesporial or oogonial cells; (2) endomitosis takes place in the emc or oocyte before or during meiotic prophase.

In obligate-apogamic ferns (*cf.* MANTON, 1950) the last of the four archesporial divisions, which usually result in sixteen spore mother cells, is subjected to a disturbance involving an interruption of the anaphase, followed by the formation of restitution nuclei. Consequently only eight spmc:s are formed, each with the tetraploid chromosome number. Sometimes the two last premeiotic divisions are changed into restitution mitoses, with the result that only four, and octoploid, spmc:s are formed. In both cases exclusively bivalents (autobivalents) are formed, in the former case with the zygoid number, in the latter case with the double zygoid number. Only spores from the former type of meiosis function at the regular propagation.

Essentially the same mechanism was found by OMODEO (1952) for parthenogenetic Lumbricidae (earthworms). The last premeiotic mitosis is a restitutional mitosis. It is characterized by strongly contracted chromosomes, which are collected undivided into one telophase nucleus. These »telophasic diplochromosomes», present in the zygoid number, develop directly into autobivalents. At metaphase I only bivalents of normal ring and rod type are present, even in triploid species. MULDAL (1952) reports similar conditions in parthenogenetic Lumbricidae. He does not describe the method of chromosome doubling but mentions quadripartite »pseudo-bivalents» with chiasmata and consisting of identical chromosomes. DARLINGTON (1949) in his John Innes report adds the information that »the paired chromosomes fall apart and divide to give diplochromosomes which arrange themselves, when their centromeres divide, like bivalents on the metaphase plate» (*l. c.*, p. 22). Thus, in this case the zygotene pairing has taken place before the endomitosis.

Some cases of parthenogenetic Planaria show still more agreement with our *Allium* cases. MELANDER (1949) reports a 21 chromosome *Polycelis* with endomitotic doubling of the chromosomes before the onset of meiosis. Consequently the first meiotic division contained 21 autobivalents. LEPORI (1949, 1950) gives a detailed report of male and female meiosis in an autotetraploid *Polycelis nigra* ($2n=24$) with premeiotic chromosome doubling. The very process of the doubling could not be observed, so it seems not to be connected with mitotic disturbances like those of OMODEO's Oligochaeta. Probably an endomitosis takes place either in the last oogonial generation, in the oocyte prior to the meiotic prophase, or during prophase before zygotene. On the female side exclusively bivalents were formed even in cases where multivalents were present on the male side. Fertilization is necessary, the male gametes functioning as activators of the egg cleavage (pseudogamy, gynogenesis).

These flatworm cases constitute the closest parallel to our *Allium* cases that we have encountered in the literature. The resemblance is especially striking with *Allium nutans*. The remarkable bivalent shape of *Allium odorum* seems to stand out as rather unique up to the present.

Reference should be made to the apomictic cockroach *Pycnoscelus surinamensis* (MATTHEY, 1945). In this animal the female meiosis starts out with the reduced number of bivalents (19 pairs). After pachytene the nucleus enters a growth phase, swelling up considerably in volume. At diakinesis and metaphase I 38 elements are counted. The same number is present in the second division. Evidently an extra chromosome reproduction takes place in connection with the volume increase. The picture is somewhat obscured by the absence of chiasma formation and the distance conjugation. MATTHEY uses the term »endoméiose» for the extra chromosome reproduction, a term which in our opinion is somewhat ambiguous. We would prefer the expression endo-duplication during the first prophase.

SUMMARY

The chromosome behaviour at male and female meiosis is analysed for the diplosporous apomict *Allium odorum*. The pairing conditions at male meiosis indicate this species to be of an autotetraploid origin, numerous structural differences having developed between the genomes.

The meiotic mechanism on the female side for compensating the lack of fertilization is unparalleled, so far, among Phanerogams. A premeiotic, probably endomitotic, doubling makes the meiotic prophase start out with the double zygoid chromosome number. Zygotene pairing does not occur, the pachytene pairs being made up from the two endomitotically formed sister strands of each chromosome. No multivalents are formed, the zygoid number of bivalents appearing. The bivalents, having had ideal conditions for their pairing, develop a high chiasma frequency, making them quite deviating in type from the ordinary *Allium* bivalent.

Since each female bivalent has developed from one single univalent chromosome without involving any homologous pairing, we propose the term *autobivalent* for this type of bivalent. Apomictic organisms with autobivalent formation have been described in the literature among ferns, planarians, earthworms, and cockroaches.

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A PHYSICIST'S COMMENTS ON SOME RECENT PAPERS ON RADIATION-GENETICS[1]

By *K. G. ZIMMER*

GENETICS DEPARTMENT, FOREST RESEARCH INSTITUTE OF SWEDEN, STOCKHOLM

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SOME 20 years ago TIMOFÉEFF-RESSOVSKY, ZIMMER and DELBRÜCK (1935) proposed a model of the gene and of the nature of its mutation. It was based on experiments with *Drosophila* mainly, though consideration had been given to the results obtained with plants such as *Antirrhinum* and maize. The main idea was to imagine the gene as a well organized and stable, molecule-like structure that can be transferred into another about equally stable state either artificially by radiation or spontaneously by accidental accumulation of sufficient thermal energy in one degree of freedom. Naturally, this model met with considerable criticism and was considered by many biologists and some chemists to be far too abstract to be of much value, though there were also papers devoted to developing the basic idea further such as the studies by CATCHESIDE and LEA (see LEA, 1946) and by MCELROY and SWANSON (1951).

On the other hand some studies appeared casting doubt on several of the results that had led to the above mentioned theoretical considerations. There were the attacks by GOLDSCHMIDT (1937, 1938) against the very concept of the gene as a unit and the contributions by STADLER and co-workers (see STADLER, 1954). We do not propose to discuss these papers here, as we cannot really add anything of import to the critical discussion given recently by MULLER (1956) and by WESTERGAARD (1957).

But, besides this one there is quite a number of other topics requiring attention. Two of the more important results underlying the model, and then widely accepted, were the following:

[1] Condensed version of a paper read at the meeting of the »Group for theoretical and applied mutation-research», November 27th 1956 at Genetics Dept., Forest Research Institute, Stockholm, Sweden.

(i) Induced back mutations to or towards the original state were believed to occur, and

(ii) the rate of spontaneous mutations had been found to be proportional to time and independant of the number of generations.

Some years later point (i) was questioned by the investigations of LEFÈVRE (1950), using *Drosophila*, but his results were considered indecisive by GUSTAFSSON (1951) and critizized quite recently by MULLER and OSTER (1956) who also quoted results in affirmation of the original findings. It should be mentioned here that in these days the *Neurospora*-technique enables very thorough investigations of this problem to be made using biochemical mutants. Actually, this has been done by GILES (1956), who found, briefly said, that though not all appearant back mutations are true ones, some are — as far as this can now be tested.

Point (ii) was re-investigated by NOVICK and SZILARD (1950) and by MOSER (1954) using bacteria and the chemostat. NOVICK and SZILARD had, in fact, hoped to show a dependance of spontaneous mutation on the number of generations, and arrived, much to their surprise, at a dependance on time only, whereas MOSER came to the conclusion that this result may be influenced by the action of selection under certain circumstances.

Consequently, it can be said that no final evidence against the original findings has been adduced so far, though more experiments using more modern methods clearly seem desirable.[1]

But there is another, third kind of evidence which, obtained somewhat later, was considered as confirming the original model: the relative mutagenic effect of equal doses of X-rays and fast neutrons. TIMOFÉEFF-RESSOVSKY and ZIMMER (1938, 1942), and during the following years many other authors (see Table 1) found a smaller efficiency of fast neutrons using the well known ClB-test in *Drosophila*. This was what had been expected from general considerations, and it also fitted in very well with the results of other investigations using other biologic materials as well as other test reactions (TIMOFÉEFF-RESSOVSKY and ZIMMER, 1947).

Fairly recently, however, new experiments have been made to test the mutagenic efficiency of fast neutrons in *Drosophila* by investigating

[1] Note added in proof: Professor H. J. MULLER has kindly drawn our attention to a short note of his: »Physiological effects on spontaneous mutation rate in *Drosophila*», Genetics (1946) 31: 225. This work, not yet published in full, seems to cast doubt on the dependence of spontaneous mutation rate on time only.

TABLE 1. *Relative mutagenic effect of fast neutrons and X-rays in Drosophila melanogaster.*

Author	Reaction	Relative efficiency
ZIMMER and TIMOFÉEFF-RESSOVSKY, 1938	Sex linked lethals	0.66 ± 0.06
ZIMMER and TIMOFÉEFF-RESSOVSKY, 1942		0.41 ± 0.06
GILES, 1943		0.73 ± 0.3
DEMPSTER, 1941		0.75
FANO, 1944		0.80 ± 0.1
DEMEREC, KAUFMAN and SUTTON, 1942		0.56 ± 0.19
MICKEY, 1954		1.8 ± 0.2
DEMPSTER, 1941	Dominant lethals	1.5
FANO, 1944		2.0
BAKER and VON HALLE, 1954		4.8 — 7.3
DEMPSTER, 1941	II-III translocations at 10 % level	1
DEMEREC, KAUFMAN and SUTTON, 1942		1
CATSCH, PETER and WELT, 1944		0.3
MULLER, 1954		1.9

sexlinked and dominant lethals as well as translocations, and the results have been quite different from the older ones, showing a considerably greater efficiency of fast neutrons as compared to equal amounts of absorbed energy in the form of X-rays (BAKER and VON HALLE, 1954; MICKEY and YANDERS, 1953; MICKEY, 1954; MULLER, 1954 a). This has led to interesting considerations on the structure of the chromosome (MULLER, 1956). We do not intend to go into these here, but would rather limit ourselves to a closer analysis of what has been done 20 years ago and what has been achieved more recently. This might be useful as no such analysis has been attempted, as far as we know, but the older results have been dealt with rather summarily in some of the newer papers.

Clearly, in such a comparison, physics in the form of irradiation-technique and of dosimetry is no less important than biology, and it is somewhat disturbing that in some of the newer papers the difference is ascribed to advances in irradiation-technique and dosimetry very generally and without stating what these advances really are. The physical part of the newer work has been published in very short form only and without reference to the older work (SHEPPARD and DARDEN, 1954). But it is not evident why the older work should be wrong, and we feel that

it would be very interesting to try and find out where the difference in the results really comes from.

There is, of course, a great number of possible sources of error to be considered such as:

(i) *Possible influence of unwanted gamma-radiation besides the desired fast neutrons.* This is, in fact, a very serious difficulty encountered in the new experiments using modern equipment. It has been tested for very carefully, and as far as we see, its possible contribution to the net effect has been taken into account adequately.

As to the older work it should be kept in mind that some knowledge of physics existed even 20 years ago and that the difficulty of dealing with admixed gamma-radiation has been fully realized. Actually, because of this, a nuclear reaction has been used in many of the experiments for the generation of neutrons which is virtually free from gamma-radiation, and the absence of the latter has been demonstrated by absorption-studies (ZIMMER, 1938, 1941; ZIMMER and TIMOFÉEFF-RESSOVSKY, 1938). Consequently, the suggestion made by several authors of newer work that the difference might be due to neglecting gamma ray effects cannot be considered very helpful.

(ii) *Improvements in dosimetry.* It has been mentioned before that these improvements have not been published in very detailed form. But the few data we have clearly indicate that the relation between the arbitrary unit of neutron dose »n», frequently used in the USA, to the »rep» has been redetermined very carefully and accurately, This determination is very useful in itself, but does not really help us very much here, for two reasons: (a) the unit »n» was not used in the older work done in Europe, because the British as well as the German workers felt from the very beginning that any arbitrary unit of dose would only add to the difficulties, and (b) the new determination of n/rep led to a value of 2.5 for the usual Victoreen-dosemeter required by definition for the measurement in n. This is a most welcome confirmation as the value of 2.5 has some time ago been arrived at as the most probable one by AEBERSOLD and LAWRENCE (1942) and independantly by HESS and ZIMMER (1944). This value has also been used extensively by various authors in evaluating early work done in the USA (see *e. g.* LEA, 1946). However, we cannot see how this good agreement could shed light on the difference we want to understand.

(iii) *Insufficient saturation of current in ion-chambers and unwanted radiation-effects in insulators.* It is well known that serious errors can be introduced into dose-measurements by not observing conditions of

saturation in ionization-chambers. But this has been tested for in detail, as described in the older work. With regard to unwanted radiation effects in insulators (induced conductivity or so-called »soakage-effects») no really thorough investigation seems to have been made in connexion with the biological experiments in question. However, it is difficult to imagine how such effects could account for the difference between older and newer work, a difference that amounts to nearly an order of magnitude in some cases. Nevertheless, some work has lately been done to test this point by detailed studies of the action of radiation on insulating materials (ROSMAN and ZIMMER, 1956 a, 1956 b; EHRENBERG and ZIMMER, 1956 a). The results obtained so far give no indication that an action of radiation on the insulators could be responsible for the differences to be explained.

The following conclusion seems, therefore, well founded: It must be considered highly unlikely that the different results obtained in the various experiments on mutagenic action of fast neutrons as compared to X-rays are due to improvements in irradiation-technique or dosimetry (see also FANO, 1954 b).

At this stage attention may be drawn to the following facts. Experiments with various biological materials using the »old fashioned type» of generating neutrons and of dosimetry have yielded smooth curves for the dependance of RBE (relative biological effect) on LET (linear energy transfer) from beta-, through gamma-, hard and soft X-rays and fast neutrons to alpha-rays. These experiments include *e. g.* sex linked lethals in *Drosophila*, killing of bacteria and of fern spores (Table 2). They cannot, of course, be considered as proving the accuracy of the older work. But they seem of interest in this connexion, as

TABLE 2. *Relative effectiveness of equal amounts of absorbed energy of various radiations on different biological effects.*

Data taken from LEA (1946) and ZIRKLE (1937).

Biological reaction	RBE for various radiations						
	Beta	Gamma	X 0.15 Å	X 2-3 Å	X 8 Å	Fast neutrons	Alpha
Sex linked lethals in *Drosophila*	1.0	1.0	1.0	0.8	—	0.4	0.3
Killing of *Bact. coli*	1.0	0.8	0.7	0.6	0.5	0.5	0.2
Killing of fern spores	1.0	—	—	—	—	1.2	3—8

the above mentioned difficulties of admixed unwanted radiation and of exact dosimetry do not exist in experiments with alpha-rays. Undoubtedly, there is a possibility for the dependance of RBE on LET to show irregularities as has been demonstrated for chromosome breakage in *Tradescantia* (see LEA, 1946). But, returning to our problem, it seems highly desirable to find out why such irregularities appear in some of the experiments with *Drosophila* and not in others.

One reason for this might be a possible dependance of mutagenic effects on dose-rate. Some, if not all, of the older experiments have been done at the low dose-rates available then and requiring hours for a few krep to be obtained, whereas many of the newer experiments have been done using powerful sources of neutrons such as a large cyclotron, a pile or even an atomic detonation, that is with much higher dose-rates. A detailed study of the possible influence of dose-rate would be highly desirable. The more so as a dose-rate effect at very high dose-rates has recently been found for the induction of sex-linked lethals in *Drosophila* by X-rays (CLARK, 1956).

Another point worth while investigation in connexion with our problem appears to be the possible contribution of non-ionizing slow protons produced by the impact of fairly slow neutrons or by slowing down faster protons. These slow protons may transfer considerable amounts of energy by direct collision with atomic nuclei (MAGEE, KAMEN and PLATZMAN, 1953; FANO, 1954 a). We do not know anything yet about the importance of this effect in bringing about changes in biological systems, though it has been demonstrated to be rather efficient in damaging crystalline materials. Should it turn out to cause an appreciable part of the biological effects of neutrons, the situation would be altered entirely as this type of energy transfer is not included in any of the dosimetric methods hitherto used.

There is, of course, quite a number of other parameters which might possibly have influenced the biological effects by fast neutrons in some of the experiments such as a strong dependance of mutagenic effects of neutrons on the neutron-energy, which is considered likely by some authors and improbable by others. Nothing much can be said about this question on theoretical grounds and the experimental evidence is practically lacking as far as *Drosophila* is concerned. Moreover, the description of experimental methods being as short as it is in some of the papers, we cannot do more at present than discuss the more obvious of the possible reasons for the discrepancy.

It should also be understood that the aims of the above discussion

are strictly limited. We did not want to discuss the evidence for and against the model of gene mutation, as proposed in 1935, in all its aspects. This would require a more detailed analysis of some new biological findings such as the clustering of mutations produced by fast neutrons (MULLER, 1954 b), the influence of oxygen pressure on biological effects of radiation as well as the mode of action of the so-called radioprotective substances. Our considerations with regard to some of these fields have recently been put forward (ZIMMER, 1956; EHRENBERG and ZIMMER, 1956 b). In the present communication we wanted to draw attention to the following points:

(i) No final evidence has been put forward yet against the occurrence of induced back mutations nor can we, in the light of contradicting and incompletely published results, consider the problem to be solved definitely, on what factors spontaneous mutation depends.

(ii) The divergency of the results of older and newer series of experiments on the mutagenic action of fast neutrons in *Drosophila* cannot, on the basis of published material, be attributed to faulty technique of irradiation nor to inadequate methods of dosimetry in any one of the experiments. The real source of this difference is not yet known. Possible lines of experimental approach are indicated.

APPENDIX

On sending our »comments» to several colleagues for further discussion, Dr C. W. SHEPPARD of Biology Division, Oak Ridge National Laboratory, has very kindly made available to us preprint copies of very interesting and relevant papers by SHEPPARD, SLATER, DARDEN, KIMBALL, ATTA, EDINGTON and BAKER (1957) and by SHEPPARD, SLATER and ATTA (1957). It is interesting that the use of ion chambers made from graphite-covered plastics in all of the newer measurements of neutron dose has now been shown to be responsible for many of the discrepancies. This source of possible error was avoided in the older series of experiments, done in Britain and Germany, where a homogeneous graphite-plastic mixture was chosen for manufacturing ion chambers (ZIMMER, 1934) and, realizing the potential risk of inaccuracy, the »skin» of the moulded parts had been removed by grinding in order to obtain optimal homogeneity of the wall material.

According to the latest results the dose values in the newer experiments on dominant lethals (BAKER and VON HALLE, 1954) have to be increased by about 30 % bringing the RBE to values of 2.9—4.5 instead

of 4.8—7.3, given before. Moreover, the dose values in the newer experiments on sex-linked lethals (MICKEY, 1954) have, according to the latest measurements, to be increased by a factor of nearly 2, bringing the RBE to about 1 ± 0.2. These changes in the newer measurements decrease considerably the discrepancies by bringing some of the newer results nearer to the older ones. Nevertheless, very serious discrepancies remain unexplained, *e. g.* in the case of II—III-translocations. Our conclusions as to the necessity of investigating in detail such problems as the dependance of mutagenic action on the speed of and on the dose-rate produced by neutrons remain, therefore, unchanged if not strengthened.

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ON ACCESSORY CHROMOSOMES IN FESTUCA PRATENSIS

V. INFLUENCE OF ACCESSORY CHROMOSOMES ON FERTILITY AND VEGETATIVE DEVELOPMENT

By *NILS OLOF BOSEMARK*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

(Received March 16th, 1957)

I. INTRODUCTION

ONE of the characteristics of accessory chromosomes or B-chromosomes in plants is that, when occurring in a low or moderate number, they apparently do not affect the phenotype of the individual plant in question. Before more detailed genetical and cytological studies had been made, accessory chromosomes were for this reason considered genetically inert.

However, the studies of accessory chromosomes in *Zea mays* (RANDOLPH, 1941; DARLINGTON and UPCOTT, 1941), *Secale cereale* (MÜNTZING, 1943, 1949, and 1954), *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941), and *Anthoxanthum aristatum* (ÖSTERGREN, 1947), have shown that, in these species, the accessory chromosomes have various genetical and cytological effects which become gradually apparent as their number is increased.

The present report concerns the effect of various numbers of accessory chromosomes on fertility and vegetative development in *Festuca pratensis*. Previous papers in this series of publications have dealt with the cytology and inheritance of the accessory chromosomes and their frequency and geographical distribution in natural populations (BOSEMARK, 1954 a and b, 1956 a and b).

II. MATERIAL AND METHODS

The material for the studies of pollen fertility consists mainly of progenies from pair crosses involving plants with low numbers of accessory chromosomes and daughter plants from eight of eleven plants with higher numbers of accessory chromosomes intercrossed in

1950. The same material has been used earlier for studies of the inheritance of the accessory chromosomes (BOSEMARK, 1954 b). Pollen fertility was measured in pollen slides mounted in equal parts of aceto-carmine and glycerine or in lacto-phenol-fuchsin. In each slide 200—250 pollen grains were counted, only well filled and morphologically normal grains being classified as good.

For the study of the vegetative development of field grown plants, the above material was largely used. The plants were cut and weighed (excluding the root system) about a week before seed ripening. In later experiments carried out in the greenhouse, daughter plants from pair crosses as well as progenies from intercrosses of a larger number of plants were used. Further details about the materials and the handling of these experiments will appear in the text.

III. OBSERVATIONS

A. Fertility

To get a general idea of the male fertility in *Festuca pratensis*, most of the plants collected in natural populations in Sweden in 1948 as well as part of the material collected in 1949 (BOSEMARK, 1956 a) were studied in the previously mentioned way. In the 1949 material, a random sample of about equal numbers of plants with and without accessory chromosomes was studied. The results are given in Table 1.

TABLE 1. *Pollen fertility in materials of Festuca pratensis collected in natural populations in Sweden.*

Year	Number of acc. chr.	Percentage of good pollen											Number of plants	Mean %
		0	10	20	30	40	50	60	70	80	90	100		
1949	0					1	9	5	12	26	92		145	87.7
	1						1		2	4	17		24	90.0
	2					1		2	1	3	7		14	83.6
	4							1	1		3		5	85.0
	7										1		1	(95.0)
1950	0	1			5	5	10	10	16	18	18		83	72.8
	1	1		1	2	2	4	3	6	15	12		46	75.4
	2		1		2		3	5	4	11	6		32	74.4
	3					1				1	2		4	(80.0)
	4				1					1	3		5	81.0

As may be seen, the pollen fertility in *Festuca pratensis* is of the kind typical of cross fertilizers (MÜNTZING, 1939). The marked difference between the two years is very likely purely modificative. There are no differences between plants with and without accessory chromosomes of the same year. Thus, the mean values for plants without accessory chromosomes in 1949 and 1950 are 87.7 and 72.8, respectively. The corresponding figures for plants with accessory chromosomes are 87.5 and 75.6. It seems unlikely from this material that the presence of accessory chromosomes in low numbers affects pollen fertility to any measurable degree. The heterogeneity of the material, however, made a more careful study desirable; therefore in 1952 the pollen fertility of progeny plants from four reciprocal crosses was studied. Because the separate progenies showed about the same distribution, only the total result is given (Table 2).

TABLE 2. *Pollen fertility in progeny plants from four reciprocal crosses.*

Number of acc. chr.	Percentage of good pollen											Number of plants	Mean %
	0	10	20	30	40	50	60	70	80	90	100		
0					2	1	3	5	30	58		99	88.6
1						3	1	4	54	70		132	89.2
2					1		1	2	37	37		78	88.7
3									1	3		4	(92.5)
4		1					1		7	4		13	80.4
Total		1			3	4	6	11	129	172		326	88.6

As in the previous material, no differences in pollen fertility between plants with and without accessory chromosomes were found. The value for plants with four accessory chromosomes is slightly lower than the others, but it is based upon a small number of plants and thus is highly influenced by one completely sterile plant, the sterility of which most probably has nothing to do with the presence of accessory chromosomes. We may conclude that the presence of one or two accessory chromosomes does not affect male fertility in *Festuca pratensis* as measured in this way.

In 1951 the pollen fertility of a number of plants with 3—15 accessory chromosomes was examined. In this material a negative correlation between pollen fertility and number of accessory chromosomes was observed. In 1952 this result was verified in a larger material consisting of progeny plants from the previously mentioned plants,

having 5—8 accessory chromosomes, which were intercrossed in 1950. The pollen fertility of altogether 391 plants with 1—16 accessory chromosomes was examined. The correlation coefficient between pollen fertility and number of accessory chromosomes was calculated for each of the progenies as well as for the total material (Tables 3 and 4).

TABLE 3. *Correlation between number of accessory chromosomes and pollen fertility.*

Mother plant	Number of daughter plants	Range of number of acc. chr.	r	t^2	P-value
K5/48: 10	44	3—10	— 0.3571	6.14	0.05—0.01
1014	27	2—12	— 0.6570	19.20	< 0.001
1151	53	2—16	— 0.3501	7.12	0.01
K14/48: 1	50	5—14	— 0.0594	—	insignificant
1016	56	2—13	— 0.2895	4.94	0.05
1108	53	3—14	— 0.4106	10.34	0.01—0.001
K11/48: 8	51	3—14	— 0.1470	—	insignificant
K14/48: 8	57	1—13	— 0.1648	—	insignificant
Total	391	1—16	— 0.2720	31.08	< 0.001

TABLE 4. *Correlation between number of accessory chromosomes and pollen fertility.*

Number of acc. chr.	Percentage of good pollen											Number of plants	Mean %
	0	10	20	30	40	50	60	70	80	90	100		
1											1	1	(95.0)
2							1			3	3	7	85.0
3		1					2	2	2	3	5	15	75.0
4		1		1	1	1		3	5	9	3	24	72.1
5			1	1	2	1	2	3	14	15	9	48	75.8
6					1	8	6	8	9	17	4	53	70.7
7		1		3	2	4	11	6	16	23	1	67	68.3
8			1		4	3	13	7	20	19	1	68	68.7
9			1		2	5	2	4	12	10		36	67.5
10		4		3	2	5	3	2	4	7		30	52.7
11					1	3	5	2	1			12	54.2
12			1		1	2	3	2	3	3		15	61.0
13			1	1			2	1	3			8	55.0
14							2	2	1		1	6	68.3
15												—	—
16									1			1	(75.0)
Total		7	5	9	16	32	52	42	91	109	28	391	68.2

All individual correlation coefficients are negative and five are significant at various levels. The correlation coefficient for the total material is highly significant. Thus, there is no doubt that plants with high numbers of accessory chromosomes have, on an average, a lower percentage of morphologically good pollen.

Experience from crosses involving plants with very high numbers of accessory chromosomes, however, indicate that the reduction in fertility in such plants is much more pronounced than is suggested by the studies now reported. In this study the disadvantage of a method which does not take into account the functioning capacity of the pollen was also demonstrated in another manner. Thus, in the daughter plants from some of the plants, a varying percentage of the pollen grains were much larger than normal, haploid ones. These large pollen grains were found to be diploid and were the result of formation of restitution nuclei after anaphase I of meiosis (BOSEMARK, 1954 a). Since diploid pollen grains were rather frequent in some plants, and it was known from the results of the crosses (BOSEMARK, 1954 b) that they function only occasionally, 200—300 well filled and morphologically normal pollen grains were measured in each slide. Haploid and diploid grains generally fell in two distinct size classes, making a relatively reliable estimation of the frequency of the latter possible. The figures

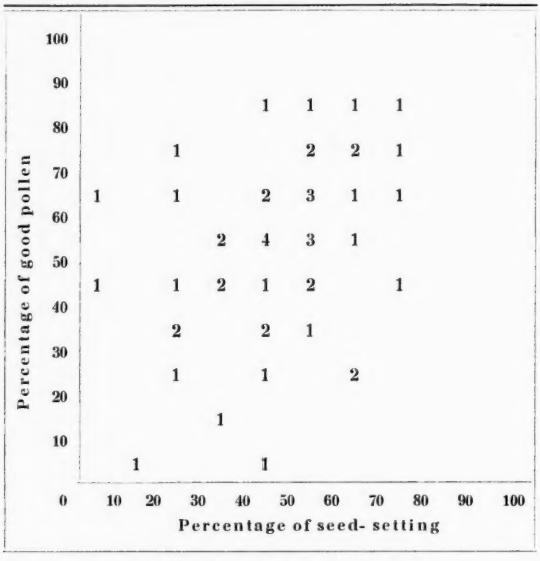
TABLE 5. *Frequency of diploid pollen grains in relation to number of accessory chromosomes.*

Number of acc. chr.	Diploid pollen grains in per cent of morphologically good pollen											
	0	0	5	10	15	20	25	30	35	40	45	50
3	1											
4	2											
5	7											
6	8											
7	14	2										
8	19	3										
9	5	2										
10	6	4	1									
11	1	2	3	1	1		1					
12	3											
13		3										
14	1		1		1	1			1			
Total	67	16	5	1	2	1	1	1				

for percentage of good pollen obtained earlier were therefore adjusted on the basis of these frequencies. Although the percentage of diploid pollen grains increases with an increasing number of accessory chromosomes (Table 5), the diploid grains alone are not responsible for the negative correlation between pollen fertility and number of accessory chromosomes demonstrated in Tables 3 and 4. A number of other factors certainly contribute to this situation; *e. g.* the frequent disturbance of the first pollen mitosis (BOSEMARK, 1954 a).

In daughter plants from the plant K 11/48: 8 with $2n=14+5$ acc., the seed-setting was determined in 200—250 flowers from the middle section of a number of panicles. The correlation between seed-setting and number of accessory chromosomes was weak ($r=-0.2061$) and insignificant. The correlation between male and female fertility in the same plants was stronger (Table 6, $r=0.3462$, $t^2=6.40$, $0.05>P>0.01$). On the basis of the more extensive studies of the male fertility, it is likely that also the seed-setting is affected in a negative direction by higher numbers of accessory chromosomes although the effect may be less marked than on the male side.

TABLE 6. *Correlation between pollen and seed fertility in progeny plants from the plant K 11/48: 8.*



$r=0.3462$, $t^2=6.40$, $0.05>P>0.01$.

From the histograms obtained in the determination of the frequency of diploid pollen grains, the average size of the haploid pollen grains could be estimated. The correlation between average pollen diameter and number of accessory chromosomes gave an r-value of +0.3631 with a $t^s=6.54$ and a P-value between 0.05 and 0.01. Thus, it is very probable that with an increasing number of accessory chromosomes the size of haploid pollen grains in *Festuca pratensis*, as in maize (RANDOLPH, 1941), increases correspondingly.

B. Speed of germination

In the above-mentioned progenies from plants with higher numbers of accessory chromosomes, preliminary observations were made on the influence of accessory chromosomes on the germination process. The seeds germinated in a germination apparatus under uniform conditions at about 15° C, and the plants were potted and numbered in order of germination. As about 45—50 per cent of the seeds from an individual germinated a few days after sowing, the number of plants within each progeny was divided into equal groups, one early and one late germinating. When the chromosome numbers of the plants had been determined, the distribution in the two groups was compared. The differences were in no case significant. For the total material, which comprised more than 650 plants, the average number of accessory chromosomes for the early and late germinating groups were 6.9 and 7.0, respectively.

In another material, consisting of equal numbers of closely related plants without, with one and with two accessory chromosomes, the seeds germinated in sterilized soil in the greenhouse. As in the previous experiment, the seedlings were marked in order of appearence. Neither in respect to the speed of germination nor in the germination capacity were there any differences between the chromosome numbers. Possibly the results could have been different if the seeds had germinated under different conditions. However, the present observations suggest that the effect of the accessory chromosomes on the germination process is very slight.

C. Earliness

In the same material as that used in the previous study earliness was determined by recording the date of flowering. Plants were considered to be flowering when the three first panicles were in flower. The period

TABLE 7. *Plant weight in progeny plants from four reciprocal crosses grown in the experimental field in 1952.*

Number of acc. chr.	Plant weight in grams																		Number of plants	Mean in gr.
	0	50	100	150	200	250	300	350	400	450	500	550	600	650	700	750	800	850		
0	1	2	3	5	9	10	20	20	21	16	10	4	4	3	2		1		131	390
1	1	4	10	9	19	25	17	15	22	20	17	15	20	11	4	2	1		212	411
2	1	3	8	7	5	8	13	18	10	15	7	6	8	2					111	375
3									1		1								2	(475)
4					2	2	2	1		1		1	1	1					11	398
Total	3	9	21	21	35	45	52	54	54	52	35	26	33	17	6	2	2		467	396

Variance ratio 1.0823, $P > 0.2$.

from the appearence of the first flowering plant until all plants were in flower was divided into two equal parts, and the distributions of the chromosome numbers in the two groups of plants obtained were compared. The average number of accessory chromosomes in the early and late groups was 6.5 and 6.9, respectively. Although this difference is very slight and insignificant, it is likely that the plants with the highest numbers of accessory chromosomes are slightly slower in development and flowering. This is suggested by the lower average weight of such plants to be described in the next section.

D. **Plant weight**

Field grown material: Progeny plants from three reciprocal crosses involving plants with low numbers of accessory chromosomes were cut and weighed before seed ripening. As the separate progenies behaved in the same way, only the total results are given (Table 7).

An analysis of variance did not reveal any heterogeneity between the different chromosome numbers. However, the very wide variation within chromosome numbers might have masked a slight effect of the accessory chromosomes in either one or the other direction. Even if this is the case, it is obvious that in the present material and under the prevailing conditions of cultivation, one or two accessory chromosomes do not affect the vegetative development to any appreciable amount.

The effect of higher numbers of accessory chromosomes, in this

TABLE 8. *Correlation between number of accessory chromosomes and plant weight.*

Mother plants	Number of daughter plants	Range of number of acc. chr.	r	t^2	P-value
14/48: 1	50	5—14	— 0.2873	4.32	0.05
1108	57	3—14	— 0.3996	10.45	0.01—0.001
1102	29	3—14	— 0.2683	2.06	0.2—0.1
1016	53	2—13	— 0.5842	26.43	$<$ 0.001
5/48: 10	45	3—10	— 0.1666	—	insignificant
12/48: 6	60	1—10	— 0.2702	4.57	0.05
14/48: 8	53	1—13	— 0.2617	3.75	0.1—0.05
1151	55	1—16	— 0.1524	—	insignificant
997	53	1—9	+ 0.0947	—	insignificant
Total	455	1—16	— 0.2576	32.20	$<$ 0.001

TABLE 9. *Correlation between number of accessory chromosomes and plant weight.*

Number of acc. chr.	Plant weight in grams																					Number of plants	Mean in gr.
	0	50	100	150	200	250	300	350	400	450	500	550	600	650	700	750	800	850	900	950			
1								1	1		1		1									4	(438)
2				1	1	1		2	2	1	3	1			1	1						14	407
3						2	1	1	5	3	2	2	1	1			1					19	430
4			1		3	2	7	3	4	3	7	5		1	3							39	393
5		2		3	4	7	8	8	9	6	4	4	1	2	1							59	338
6			1	3	1	6	8	6	11	9	8	8	4	3	1	1	2	1				73	414
7		3	2	2	4	8	5	18	5	6	3	6	4	1	2		1			1		71	355
8			3	2	5	12	9	11	7	6	5	5	2	3								70	333
9				3	6	4	5	8	7	5	2	2		2								44	325
10			1	4	1	4	8	7	2	3	1		1									32	288
11		1		1	1					1	1											5	245
12			3			1	1	2	2	1	1											11	275
13				1	1	4	1	1		1												9	245
14					1	1		2														4	(263)
15																						—	—
16					1																	1	(175)
Total		6	11	20	29	52	53	70	55	45	38	33	14	13	8	2	4	1		1		455	353

respect, was studied in roughly the same material as that used for the study of the pollen fertility. The correlation between plant weight and number of accessory chromosomes was calculated for the separate progenies as well as for the total material (Table 8).

The predominantly negative correlation coefficients for the individual progenies and the highly significant correlation coefficient for the total material show that plants with high numbers of accessory chromosomes have, on an average, a lower plant weight. As indicated by the correlation coefficients, and by Table 9, where the individual plant weights for the total material are presented, the deleterious effect of even the highest numbers of accessory chromosomes on the vegetative development must be considered relatively moderate. It is, however, very likely that the level at which the effects of duplicated accessory chromosomes begin, varies with the genotypic constitution of the material.

Experiments in the greenhouse: Results of the kind now described were to be expected as there is a sparse occurrence of plants with higher numbers of accessory chromosomes in natural populations (BOSEMARK, 1956 a), in spite of the ease with which such plants can be produced in artificial crosses (BOSEMARK, 1954 b). Of considerably greater interest than the negative effects of high numbers of accessory chromosomes would be information on the effect of the numbers prevailing in natural populations, *viz.* one and two. In the previously mentioned experiments with low numbers of accessory chromosomes, there was no evidence of either a negative or a positive effect. In an attempt to study this question under more favourable conditions, experiments were carried out in the greenhouse in beds with sterilized soil.

The seeds were sown in rows, 5 cm within and 10 cm between rows. To avoid attacks by fungi, the plants had to be clipped about once a month. At each harvest the individual plants were weighed; after the third harvest they were potted, root-tips fixed and the chromosome numbers determined. In a preliminary experiment in 1952, progeny plants from two reciprocal crosses (14+1 acc.)×14 and (14+2 acc.)× ×(14+1 acc.) were used. In the first mentioned cross, in each harvest, the average weight for plants with one and two accessory chromosomes was slightly higher than for plants lacking such chromosomes. In Table 10 are given the individual plant weights for all harvests taken together.

The difference between chromosome numbers was tested by an analysis of variance as well as by calculating the correlation between

TABLE 10. *Pooled plant weights from three successive harvests. The first experiment in the greenhouse with progeny plants from the cross 493×494 (1×0 and recipr.).*

Number of acc. chr.	Plant weight in grams																						Number of plants	Mean in gr.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
0		1	5	1	7	6	11	17	15	20	28	8	7	5	3	2		1					137	7.99
1					3	2	4	9	11	15	8	9	5	5		1	1					1	74	8.80
2				1				2	4	2	4		1	1	1		1						17	9.03
Total		1	5	2	10	8	15	28	30	37	40	17	13	11	4	3	2	1				1	228	8.33

$r=0.1391$, $t^2=4.46$, $0{,}05 > P > 0{,}01$.

Analysis of variance:

Source of variation	Df	Sum of squares	Mean square	Variance ratio	P-value
Between chr. numbers	2	40.4005	20.2003	2.387	0.2—0.05
Between 0 acc. and 1 + 2 acc.	1	39.6557	39.6557	4.686	0.05—0.01
Between 1 acc. and 2 acc.	1	0.7448	0.7448	11.361	insignificant
Within chr. numbers	225	1903.9284	8.4619		
Total	227	1944.3289			

number of accessory chromosomes and plant weight. As may be seen, the statistical significance is not convincing, but since the result also in the other cross suggested an optimum for plants with two accessory chromosomes, it was considered possible that a stimulating effect of the accessory chromosomes on the vegetative development was really at hand.

To obtain better information on this point a new experiment was carried out in the summer of 1953. The material consisted of the progenies from seven sister plants intercrossed in 1952. Four of the plants had $2n=14$, two had $2n=14+2$ acc., and one had $2n=14+3$ acc. From each of the plants the same number of seeds were taken out and carefully mixed. A random sample from the mixture showed that the chromosome numbers 14, $14+1$ acc., and $14+2$ acc. were present in about equal numbers. The seeds were sown in the previously mentioned manner. After one and a half month, the first harvest was taken, followed by two more harvests with a month's interval. After the third harvest, the experiment was discontinued and the chromosome numbers of the plants determined. The results are given in Tables 11 and 12. Due to the wide variation in plant weight, especially in the last harvest, only the average plant weights for the different chromosome numbers and harvests can be given.

At the first harvest, there are no differences between plants without, with one or with two accessory chromosomes. The difference between this group as a whole and the plants with from 3—5 accessory chromosomes, however, is highly significant with a P-value below 0.001. In the second harvest, plants with one accessory chromosome have a slightly higher average plant weight than have plants without. The previously mentioned difference at the first harvest is still present at this harvest. At the third harvest, the average weights of the plants with one and two accessory chromosomes is markedly higher than for the plants without accessories. However, an analysis of variance of the total material did not indicate significant differences between the different chromosome numbers with respect to plant weight. As the material was constituted with the purpose of comparing the plant weights of individuals without and with one and two accessory chromosomes it may nevertheless be justified to test the former category against the two others. This test gave a quotient of 5.040 with a P-value between 0.05 and 0.01.

There is thus reason to believe, as was the case also in the previous experiment, that plants with one and two accessory chromosomes, at

TABLE 11. *Average plant weights in the experiment in the greenhouse, using progeny plants from seven intercrossed sister plants.*

Number of acc. chr.	Average plant weights								Number of plants
	I. harvest		II. harvest		III. harvest		Total harvest		
	grams	rel. values	grams	rel. values	grams	rel. values	grams	rel. values	
0	1.73 ± 0.195	(100)	5.15 ± 0.304	(100)	7.51 ± 0.562	(100)	14.41 ± 0.444	(100)	287
1	1.73 ± 0.190	(100)	5.37 ± 0.256	(104)	8.46 ± 0.510	(113)	15.63 ± 0.397	(108)	306
2	1.64 ± 0.180	(95)	5.07 ± 0.259	(98)	8.20 ± 0.518	(109)	14.94 ± 0.399	(104)	311
3	1.45 ± 0.282	(84)	4.40 ± 0.400	(85)	7.68 ± 0.839	(102)	13.43 ± 0.622	(93)	82
4	1.35 ± 0.342	(78)	4.53 ± 0.516	(88)	7.77 ± 1.081	(103)	13.91 ± 0.822	(97)	66
5	1.36 ± 0.633	(79)	4.81 ± 1.023	(93)	7.86 ± 1.841	(105)	13.92 ± 1.503	(97)	19

TABLE 12. *Variance ratios for different comparisons in Table 11.*

I. harvest		II. harvest		III. harvest		Total harvest	
Source of variation	Variance ratio	Source of variation	Variance ratio	Source of variation	Variance ratio	Source of variation	Variance ratio
Between all chr. numbers	5.783***	Between all chr. numbers	3.265**	Between all chr. numbers	1.544	Between all chr. numbers	1.943
0 + 1 + 2/3 + 4 + 5	26.534***	0 + 1 + 2/3 + 4 + 5	13.074***	0/1 + 2	5.040*–**	0/1 + 2	7.937**–***
				1/2	1.492	1/2	2.248

*=significance at the 0.05 level. **=significance at the 0.01 level. ***=significance at the 0.001 level.

least under certain conditions, have a more rapid vegetative development than comparable plants without such chromosomes. It should also be observed that at the last harvest the plants with 3—5 accessory chromosomes were no longer inferior to the plants without accessory chromosomes. The experiment also indicates the possibility that plants with accessory chromosomes are less sensitive to frequent clipping.

However, the material used for this study has certain weaknesses. The chromosome numbers of the intercrossed sister plants were chosen in such a way as to ensure that plants without accessory chromosomes had to take part also in the formation of progeny plants with one and two accessory chromosomes. Yet it cannot be excluded that recessive genes, which when homozygous lower the vitality, have met more frequently in some chromosome numbers than in others.

To avoid the possibility of complications of the above mentioned type in the following experiment, progeny plants from a single pair cross (14+1 acc.)×(14+1 acc.) were used. All the seeds were taken from only one of the parental plants. The experiment was started in August, 1954 with the intention of keeping the material over the winter and to utilize the whole vegetation period of 1955. However, the material was badly damaged during the winter, and only the data for the first harvest, taken in the fall of 1954, are reliable. In the spring of 1955, the seed from the other parental plant was sown out and during the summer four harvests were taken (Tables 13 and 14).

As may be seen from the tables the result of this experiment hardly confirms the conclusions drawn from the two previous ones. At the first two harvests the plants with accessory chromosomes appear to be inferior to those without accessories and only at the third and fourth harvest do plants with two accessory chromosomes surpass plants without such chromosomes. It should be pointed out that in this experiment, as in the previous ones, there is a strong correlation between the individual plant weights from harvest to harvest. This indicates that at least within chromosome numbers, the effect of the clipping is fairly uniform. Thus, in spite of the wide variation, the average plant weights must be considered rather reliable even for the later harvests.

In experiments of the type now described, the necessity of determining the chromosome numbers of all the individual plants puts a limit to the size as well as the number of experiments that can be carried out. At the same time as the above-mentioned experiment was going on, another one was started, lacking this complication. From two reciprocal crosses (14+1 acc.)×14, which for convenience are here called

TABLE 13. *Average plant weights in the experiment in the greenhouse, using progeny plants from a pair cross (14+1 acc.)×(14+1 acc.).*

Number of acc. chr.	Average plant weights								Number of plants
	I. harvest		II. harvest		III. harvest		IV. harvest		
	grams	rel. values	grams	rel. values	grams	rel. values	grams	rel. values	
0	3.65 ± 0.109	(100)	4.45 ± 0.117	(100)	4.09 ± 0.163	(100)	4.27 ± 0.194	(100)	523
1	3.54 ± 0.095	(97)	4.27 ± 0.115	(96)	3.85 ± 0.163	(94)	4.04 ± 0.201	(95)	431
2	3.47 ± 0.158	(95)	4.34 ± 0.188	(98)	4.25 ± 0.274	(104)	4.55 ± 0.330	(107)	151
3	3.31 ± 0.232	(91)	4.03 ± 0.327	(91)	3.58 ± 0.453	(88)	3.83 ± 0.535	(90)	50

TABLE 14. *Variance ratios for different comparisons in Table 13.*

I. harvest		II. harvest		III. harvest		IV. harvest	
Source of variation	Variance ratio	Source of variation	Variance ratio	Source of variation	Variance ratio	Source of variation	Variance ratio
Between all chr. numbers	2.412*	Between all chr. numbers	2.612*	Between all chr. numbers	3.745**	Between all chr. numbers	2.936*
0/1 + 2	6.240*–**	0/1 + 2	6.135*–**	0/1 + 2	4.215*	0/1 + 2	0.209
				0/1	4.556*	0/1	2.914
				0/2	1.199	0/2	2.021

A×B and C×D individual progeny plants were taken out. From the A×B cross, 30+30 plants with $2n=14$; while from the C×D cross, 30 plants with $2n=14$ and 30 plants with $2n=14+2$ acc. were chosen. The plants from the A×B cross were designated A1—A30 and B1—B30. Plants without accessory chromosomes from the C×D cross were called C1—C30 and those with two accessory chromosomes D1—D30. With the thirty A-plants the same number of pair crosses were done using the C-plants as the other parent; in the same way, the B-plants were combined with the D-plants. The seed from the A×C crosses will all give plants with $2n=14$, whereas in the B×D crosses the seed harvested on B-plants will give about 10 % plants with $2n=14$, 20—30 % plants with $2n=14+1$ acc., and 60—70 % plants with $2n=14+2$ acc. Finally, the seed harvested on the D-plants will give 5—10 % plants with $2n=14$ and 90—95 % plants with $2n=14+1$ acc. (BOSEMARK, 1954 b).

A random sample of about 100 progeny plants from the D-mothers showed that 94 % of these plants had $2n=14+1$ acc., and it is very likely that also the chromosome numbers of the progeny plants from the B-mothers came close to expectation. Twenty-seven of the A×C crosses gave an abundance of seeds. Of the B×D crosses, twenty-four gave satisfactory seed-setting in both directions. From each of the twenty best A×C crosses, thirty seeds were taken out and mixed carefully, thus constituting a group with $2n=14$. In the same way thirty seeds from each of the B and D plants taking part in the twenty best B×D crosses formed groups with predominantly $2n=14+1$ acc. and $2n=14+2$ acc. For convenience, the two last mentioned groups will subsequently be referred to as plants with one and plants with two accessory chromosomes. From each of the three groups twenty-two rows were sown in a greenhouse bed. Rows with different chromosome numbers alternated regularly, but in such a way that rows with a certain chromosome number were surrounded by the two others equally often. Four harvests were taken with about the same intervals as in previous experiments. The results are given in Tables 15 and 16.

At the first harvest, plants with one accessory chromosome are clearly inferior to plants without accessory chromosomes, whereas plants with two accessory chromosomes are superior to both. In the second harvest, the difference between plants without and plants with one accessory chromosome is smaller than in the first harvest, whereas the difference between the former and plants with two accessory chromosomes has increased; the relative values are 100 and 109. At the third

TABLE 15. *Average plant weights in the experiment in the greenhouse with the synthetic populations with 0, 1, and 2 accessory chromosomes, respectively.*

Number of acc. chr.	Average plant weights								Number of plants
	I. harvest		II. harvest		III. harvest		IV. harvest		
	grams	rel. values	grams	rel. values	grams	rel. values	grams	rel. values	
0	1.55 ± 0.066	(100)	3.87 ± 0.137	(100)	4.52 ± 0.169	(100)	3.60 ± 0.170	(100)	460
1	1.32 ± 0.066	(85)	3.62 ± 0.143	(94)	4.45 ± 0.173	(98)	3.63 ± 0.169	(101)	462
2	1.65 ± 0.052	(106)	4.22 ± 0.123	(109)	4.95 ± 0.153	(110)	3.99 ± 0.157	(111)	461

TABLE 16. *Variance ratios for different comparisons in Table 15.*

I. harvest		II. harvest		III. harvest		IV. harvest	
Source of variation	Variance ratio	Source of variation	Variance ratio	Source of variation	Variance ratio	Source of variation	Variance ratio
Between all chr. numbers	34.256***	Between all chr. numbers	20.102***	Between all chr. numbers	11.213***	Between all chr. numbers	7.262***
0/1	35.227***	0/1	7.343**	0/1	2.372	0/1	0.141
0/2	4.085*	0/2	13.009***	0/2	13.958***	0/2	12.050***

harvest, the plants with one accessory chromosome have practically taken in the start of the plants without accessory chromosomes; the relation between the latter and plants with two accessory chromosomes being about the same. Thus, the relative values for the three chromosomes numbers are 100, 98, and 110. The difference between plants without and with two accessory chromosomes is significant at the 0.001 level. At the last harvest, finally, the plants with one accessory chromosomes have even surpassed the plants without accessory chromosomes, and the difference between the latter and plants with two accessory chromosomes is even greater than at the previous harvest. The result of this experiment is thus on the whole the same as in the two first ones and indicates a stimulating effect of one and two accessory chromosomes on the vegetative development.

The value of this last experiment rests on the assumption that the genotypic variation in the three groups of plants is the same, and that they, on an average, differ only with respect to the number of accessory chromosomes present. It might be argued that the behaviour of the plants with one accessory chromosome at the first harvest seems incompatible with such an idea. Plants with one and two accessory chromosomes, however, came from the same reciprocal crosses, and their average genotypes must be considered largely to be the same. Furthermore, it is rather unlikely that the effect of one accessory chromosome should be more deleterious than that of two. It is, therefore, probable that the slower initial development of the plants with one accessory chromosome compared to that of the others is due to some other factor; *e. g.* a slight difference in seed quality.

It should be mentioned that soon after the germination, some eighty plants were damaged by snails and had to be replaced by other plants which had been sown in wooden boxes at the same time as the experiment. Unfortunately, no records were taken of the number of damaged plants within the different chromosome numbers. It is thus possible that more plants with one accessory chromosome were replaced than were there in the other groups. However, there were no noticeable differences in growth between plants which had been transplanted and the others, but there remains the chance that the growth of the former was slightly hampered. Be this as it may, the fact remains that in this material, after the first harvest, the growth rate of plants with accessory chromosomes, especially those with one, is higher than that of plants without accessory chromosomes.

IV. DISCUSSION

The reduced fertility and vigour of plants with higher numbers of accessory chromosomes now demonstrated in *Festuca pratensis*, parallels previous observations on accessory chromosomes in other grasses (RANDOLPH, 1941; DARLINGTON and THOMAS, 1941; MÜNTZING, 1943, 1954; ÖSTERGREN, 1947; and GARBER, 1950). The level at which these effects become apparent varies between different species and very likely also between different genotypes within a species (RANDOLPH, 1941). Thus in maize, at least 10, often 15—20, accessory chromosomes have to be present to give a noticable reduction in seed-setting and plant vigour. In the Swedish rye material studied by MÜNTZING (1943), however, a negative effect on seed-setting could be demonstrated in plants with so few as two accessory chromosomes. In the present material of *Festuca pratensis*, it has not been possible to demonstrate any negative effects on fertility and vegetative development of the low numbers of accessory chromosomes, *viz.* one and two, prevailing in natural populations. On the contrary, some experiments clearly suggest that under certain conditions low numbers of accessory chromosomes may stimulate vegetative development. Whatever are the effects of accessory chromosomes one should, however, not expect them to be unconditional and universally present. Studies on the frequency and geographical distribution of accessory chromosomes in natural populations of *Festuca pratensis* (BOSEMARK, 1956 a) suggest that ecologic conditions as well as the genotypic constitution of a population influences both the transmission and the genetic effect of the accessory chromosomes.

The lack of uniformity in behaviour of the different materials used in the experiments in the greenhouse likewise speaks in favour of a decisive role of the genotype on the effect of the accessory chromosomes. Thus, the plants in the first experiment in the greenhouse (Table 10) originated from a reciprocal cross K 87—88/50, involving plants 493 and 494, collected in a natural population. The seven sister plants intercrossed to produce the material used in a later experiment (Table 11) originated from the reciprocal cross K 85—86/50, which involved the plants 492 and 493. In the last experiment, finally, the primary cross C×D was actually K 85—86/50. In all experiments which suggest a positive influence of the accessory chromosomes on the vegetative development, plant 493 has thus contributed not only the accessory chromosomes, but also part of the genotypic constitution of the material. The behaviour of these materials is also largely uniform. In the

experiments which did not show any positive effect of the accessory chromosomes, the material originated from a pair cross involving plants which were not related to any of the plants taking part in the previously mentioned crosses.

Although only the negative effects of high numbers of accessory chromosomes on fertility and vegetative development in *Festuca pratensis* are indisputable, it may be concluded that the effect of one and two accessory chromosomes on the vegetative development may be either positive, neutral or even negative. Which one of these possibilities, that will be realized will depend on the genotypic constitution of the material and the prevailing environmental conditions. Such an idea is not supported by the results obtained by MÜNTZING (1943) for Swedish rye and is incompatible with the hypothesis of the parasitic nature of accessory chromosomes (ÖSTERGREN, 1947). MÜNTZING (1954), however, in a field trial compared two groups of materials derived from a primitive rye variety from Transbaikal. In one of the groups, the average number of accessory chromosomes was as high as 3.11, whereas in the other group the average was 0.61. The kernel yield of the plants with the higher numbers of accessory chromosomes amounted to only 52 per cent of that of the other group. On the other hand the straw weight in the low-yielding group was significantly higher than in the high-yielding. MÜNTZING does not consider the latter difference to be due to a stimulating effect of the accessory chromosomes on the vegetative development but rather that the vegetative development has been favoured by the reduced kernel production. As a result it is concluded that the total action of the accessory chromosomes is at least predominantly deleterious also in the Transbaikal variety.

Although the vegetative development during seed-development is highly influenced by climatic conditions, previous data from Swedish rye material (MÜNTZING, 1943) do not support this explanation. As about 60 per cent of the plants in the group with the low kernel yield had at least four accessory chromosomes (see MÜNTZING, 1954, Table 2) this material should not be compared with the balanced Transbaikal population, where plants with more than two accessory chromosomes constitute only about 10 per cent of the plants with accessory chromosomes. On the assumption of exclusively negative effects of accessory chromosomes in rye, and with the results of the experiments with Swedish varieties in mind, it is reasonable to suggest that plants with four accessory chromosomes, at the time of flowering, should be less well developed than plants without and with a low number of such chro-

mosomes. Now the proposed effect of the reduced kernel production does not set in until some time after flowering. Thus, the vegetative growth during seed development should have been capable of compensating not only for a probable inferiority of the plants with high numbers of accessory chromosomes at the time of flowering, but also to shift this inferiority to a significant superiority. The relatively moderate frequency of accessory chromosomes in the balanced Transbaikal population certainly rules out the possibility that the net effect of the high numbers present in the group with the low kernel production is advantageous. However, it cannot be excluded that, in the Transbaikal population, a rather slight negative effect of one and two accessory chromosomes on the seed-setting is counteracted by a stimulating effect on the vegetative development. In this manner a balance may be achieved. The level at which this balance is reached should then depend upon the genotypic constitution of the material, the environmental conditions, and the effectiveness of the mechanism for numerical increase of the accessory chromosomes. The great value of the non-disjunction mechanism would lay in the ease and rapidity with which it makes possible the regulation of the frequency of accessory chromosomes in a population in relation to changes in genotypic constitution and environment. Even when the total effect of the accessory chromosomes is entirely negative, the mechanism is likely to secure a low frequency of such chromosomes in the population. This may be what is observed in most highbred rye varieties in Europe, where accessory chromosomes are seldom found and appear to have only negative effects.

Experimental evidence for a positive effect of accessory chromosomes on the vegetative development have earlier been obtained in *Centaurea Scabiosa* by FRÖST (1954). Progeny plants from a single reciprocal cross grown in a greenhouse bed were cut and weighed after three months of growth. Plants with one and two accessory chromosomes were found to have a significantly higher average plant weight than sister plants lacking such chromosomes. Experiments carried out in the field indicate that this effect in *Centaurea* is not restricted to the early stages of development.

From the reasoning presented here, it should not be inferred that the author believes that the genetic effect of accessory chromosomes is uniform for all species. It is quite possible that in some species the over all frequency of accessory chromosomes is at present steadily decreasing due to predominantly negative effects. It is, however, equally

possible that in others, it is instead increasing. Studies of the frequency and geographical distribution of the accessory chromosomes might help to throw light on this question for each particular species. In all species in which the accessory chromosomes have a wide geographical distribution, and in which they are reasonably common, at least in certain areas, and in which they have a common morphology, they are very likely old and must be considered as existing in some kind of balanced condition.

Although it might be impossible not only to determine how the accessory chromosomes have arisen, but also what their specific genetic effects in the different species are, the author considers that the steadily increasing number of species in which accessory chromosomes are found, and the often rather complicated cytological mechanisms which such chromosomes have often evolved, point to this evolution having been promoted by a selective advantage of individuals carrying moderate numbers of such chromosomes. Lack of experimental evidence of positive effects of accessory chromosomes in materials where adaptability cannot be tested and which have a narrow genetic basis should not be generalized and considered as proof of entirely negative effects. Experiments with materials of diverse origin, cultivated under diverse but fully controlled conditions, are necessary to settle this problem for each species under question.

SUMMARY

(1) The influence of various numbers of accessory chromosomes upon fertility and vegetative development in *Festuca pratensis* was studied.

(2) No noticeable reduction in pollen fertility was found in plants having one and two accessory chromosomes. Plants with high numbers of accessory chromosomes have, on an average, a lower percentage of morphologically good pollen. With an increase in number of accessory chromosomes, pollen fertility decreases correspondingly.

(3) Part of the reduction in pollen fertility in some plants with high and very high numbers of accessory chromosomes is probably due to formation of restitution nuclei at anaphase I of meiosis. The diploid pollen grains thus formed are morphologically good but function only occasionally.

(4) Also seed-setting appears to be affected in a negative direction by high numbers of accessory chromosomes.

(5) The effect of one and two accessory chromosomes on the vegetative development appears to be partly dependent upon the genotypic constitution of the material. Thus, in some materials, the results indicate a slight stimulating effect of such chromosomes on the vegetative growth. In other experiments, however, no such effect could be observed.

(6) Plants with high to very high numbers of accessory chromosomes have, on an average, a lower weight than plants without and with a few such chromosomes. As in the case of pollen fertility, the effect of the accessory chromosomes is cumulative.

(7) No effect of even the highest numbers of accessory chromosomes on the spead of germination and germination capacity could be observed. The results are, however, only preliminary.

(8) It is concluded that the genetic effect of accessory chromosomes in *Festuca pratensis*, and probably other species, as well, is partly governed by the genotypic constitution of the material and partly by the prevailing environmental conditions.

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FURTHER STUDIES ON ACCESSORY CHROMOSOMES IN GRASSES

By *NILS OLOF BOSEMARK*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN
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I. INTRODUCTION

THE number of plant as well as animal species which have been found sometimes to have accessory chromosomes or B-chromosomes in addition to the ordinary chromosome complement is steadily increasing. In the last edition of the Chromosome Atlas of Flowering Plants (DARLINGTON and WYLIE, 1955), B-chromosomes are reported for a total of 150 species from 82 genera representing 29 families. In relation to the large number of species listed in the atlas, this is a small number; but if we take into consideration that for most of these species, very few individuals have been studied, the figures are more impressive. Thus one can safely conclude that in the future numerous new examples will be added. Within the *Gramineae* are listed 30 species from 18 genera where accessory chromosomes have been found, and thus 20 per cent of all cases are to be found within this family. So far the most intensive research on accessory chromosomes in plants has also been made in grasses.

The well known studies of accessory chromosomes in *Zea mays* by, among others, LONGLEY (1924, 1927, 1938), RANDOLPH (1928, 1941), DARLINGTON and UPCOTT (1941), and ROMAN (1947, 1948, 1950) and in *Secale cereale* by MÜNTZING and co-workers (MÜNTZING, 1943, 1944, 1945, 1946 a, 1948 a and b, 1949, 1950, 1951, and 1954; MÜNTZING and LIMA-DE-FARIA, 1949, 1952; HÅKANSSON, 1948 a; and LIMA-DE-FARIA, 1948) together with the works in *Sorgum purpureo-sericeum* (JANAKI-AMMAL, 1940; DARLINGTON and THOMAS, 1941), *Anthoxanthum aristatum* (ÖSTERGREN, 1947), *Poa alpina* (MÜNTZING, 1946 b, 1948 c, 1949, 1954; MÜNTZING and NYGREN, 1955 b; HÅKANSSON, 1948 b; and NYGREN, 1955), *Festuca pratensis* (BOSEMARK, 1954 a and b, 1956 a and b, 1957) and *Phleum phleoides* (BÖCHER, 1950; BOSEMARK, 1956 c) have shown that although the accessory chromosomes in these grasses have many characteristics in common, they deviate markedly in some respects.

Thus, in all but one species, the accessory chromosomes have been found capable of doubling their number in every generation by an essentially similar mechanism of mitotic non-disjunction, whereas two species are exceptions in so far that the accessory chromosomes are present only in the »germ line» and are largely eliminated from the vegetative tissue.

However, to enable us to form a firmer idea of the significance of accessory chromosomes in plants, further comparative studies in a relatively large number of species are needed. In the present paper, the cytology and transmission of accessory chromosomes in six more grass species is described. The material has not always been large enough for a detailed study, and in most of the species further work is planned.

II. MATERIALS AND METHODS

In 1954, the author collected plants of *Phleum phleoides* in southern Sweden, with the purpose of studying the frequency of accessory chromosomes in natural populations of this species (BOSEMARK, 1956 c). At the same time, collections were made of a number of other grass species with the hope of finding some plants with accessory chromosomes. The species to be sampled were not choosen completely at random, but preference was given to species or genera for which »fragment» chromosomes had been reported in the literature.

In all cases, established population plants were collected. Cultivated land was avoided as far as possible and the plants were taken completely at random within the area inhabited by the population. The number of plants from each population is small; however, with the knowledge of the geographical distribution of accessory chromosomes in *Festuca pratensis* (BOSEMARK, 1956 a), it was considered better to have a few plants from a larger number of populations than many plants from one or two populations. In Table 1 are listed the different species, the number of plants and populations and the chromosome numbers found.

As may be seen, in five of the ten species sampled, plants with accessory chromosomes were found. The fact that such plants were not found in the remaining species should of course not be taken to mean that accessory chromosomes are non-existant here. For all species, possibly with the exception of *Anthoxanthum odoratum*, the number of plants and populations sampled is far too small to have any significance in that respect.

TABLE 1. *Material collected in natural populations in southern Sweden in 1954.*

Species:	Number of plants	Number of populations	Chr. numbers
Poa trivialis L.	26	4	14 + 0 − 4 acc.
Briza media L.	58	8	14 + 0 − 2 acc.
Cynosurus cristatus L.	60	9	14
Dactylis Aschersoniana GRAEBN.	17	1	14
Koeleria glauca (SCHRAD.) DC.	65	6	14
Holcus lanatus L.	73	9	14 + 0 − 2 acc.
Alopecurus pratensis L.	70	9	28 + 0 − 6 acc.
Alopecurus geniculatus L.	15	3	28
Phleum nodosum L.	40	5	14 + 0 − 4 acc.
Anthoxanthum odoratum L.	148	23	20

Besides the species with accessory chromosomes listed in Table 1, *Festuca arundinacea* also, will be treated in this paper. The material of this species originates from seeds collected in England in 1951.

All chromosome counts were made in root-tips fixed in chrome-acetic-formalin and sectioned at 13—15 μ. The slides were stained in crystal violet. For the studies of meiosis and pollen mitosis, panicles were fixed in acetic-alcohol (1:3) and stored in a deep-freezer. Meiosis was studied in aceto-carmine and Feulgen squashes, the first pollen mitosis in aceto-carmine squashes. In studying the transmission of the accessory chromosomes, crosses were made by isolating the parental plants in special isolation cages in a green-house. With this method, it cannot be avoided that a few plants are obtained which are the result of self-fertilization. As the main purpose of the crosses has been to reveal the presence of possible mechanisms for numerical increase of the accessory chromosomes, a few such plants will, however, generally not obscure the results. In most cases, control isolations also were made at the same time. Most of the plants isolated proved to be highly self-sterile.

III. OBSERVATIONS

A. Festuca arundinacea

The chromosome number of *Festuca arundinacea* SCHREB. has been determined to $2n=42$ by several workers, among others, LEVITSKY and KUZMINA (1927), PETO (1934), NILSSON (1940), MYERS and HILL (1947),

and CROWDER (1953). LEVITSKY and KUZMINA also found two varieties with $2n=70$. *Fragment chromosomes* or *supernumerary chromosomes* were observed in a few plants by PETO, MYERS and HILL, and CROWDER. The size and appearence of these chromosomes correspond very well to the accessory chromosomes to be described here and thus are very likely identical.

1. Material collected

While collecting seed from natural populations of *Festuca pratensis* in England in 1951, the author also sampled a population of *Festuca arundinacea* near Sandwich, Kent. The population plants were not distinctly different from *F. pratensis* in size and robustness, and in 1952 a random sample of seeds from about 30 panicles were sown for a control of the chromosome number. All seedlings had 42 normal chromosomes; and on the clayey soil in the experimental field, they were also easily distinguishable from *F. pratensis*. However, some of the plants had accessory chromosomes in addition to the ordinary ones (Table 2).

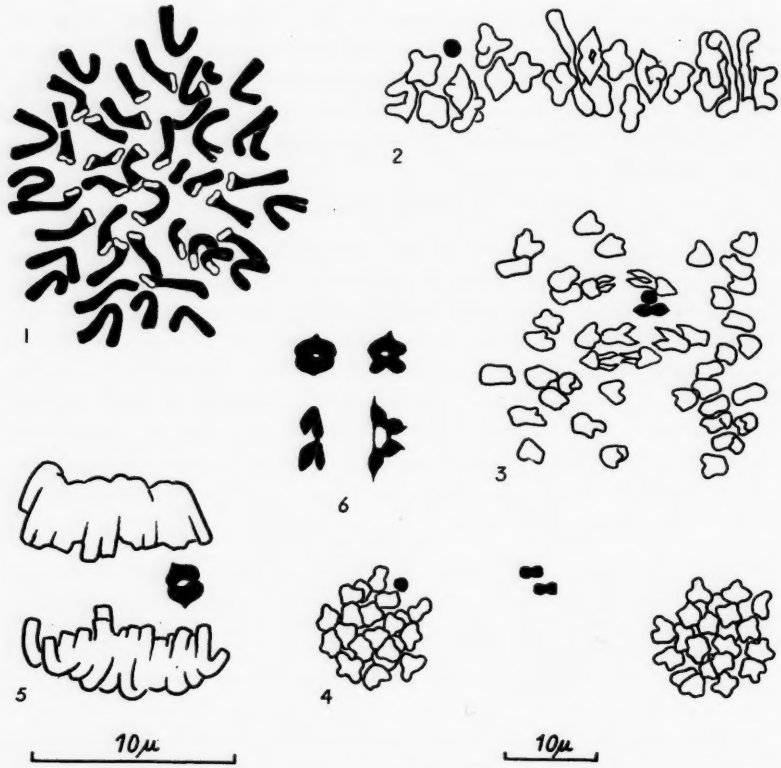
TABLE 2. *Frequency of accessory chromosomes in plants of Festuca arundinacea raised from seed collected near Sandwich in England.*

Number of plants with respective numbers of accessory chromosomes				Total number of plants	% plants with acc. chr.
0	1	2	3		
10	6	4	2	22	54.5

2. Cytological observations

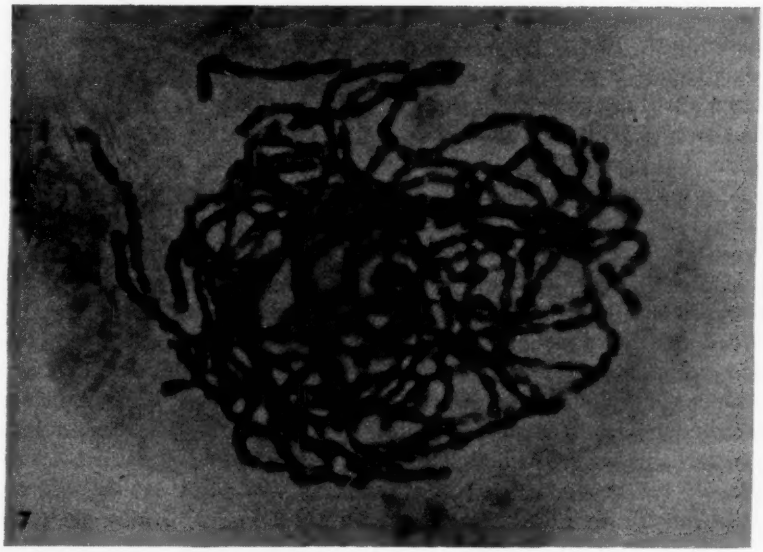
The accessory chromosomes have an approximately median centromere, and in root-tip metaphase plates their length is about $^1/_4$ of the average of the ordinary chromosomes (Fig. 1). In resting nuclei and at early prophase in root-tips, they show positive heteropycnosis. The number of accessory chromosomes seems to be constant in the individual plant, the counts in root-tips and anthers always corresponding. Thus the appearence and behaviour of the accessory chromosomes of *F. arundinacea* in root-tip mitoses completely agrees with that of the standard type of accessory chromosome in *F. pratensis* (BOSEMARK, 1954 a).

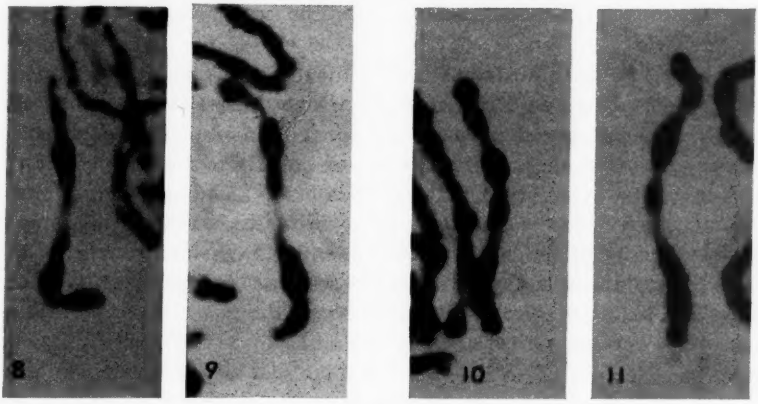
Meiosis was studied in six plants with 1—3 accessory chromosomes and in two plants without accessories. At pachytene the morphology



Festuca arundinacea. — Fig. 1. Root-tip metaphase plate from a plant with $2n=42+2$ acc. — Fig. 2. Metaphase I in a plant with $2n=42+1$ acc. — Fig. 3. Anaphase I in a plant with $2n=42+3$ acc. — Fig. 4. Late anaphase I with two lagging univalents in the same plant. — Fig. 5. Anaphase of the first pollen mitosis in a plant with $2n=42+2$ acc. — Fig. 6. Configurations of acc. chr. at anaphase of the first pollen mitosis, separately drawn.

of paired accessory chromosomes in *F. arundinacea* was compared to that of the standard type in *F. pratensis* (Figs. 7—11). At comparable stages of contraction at early pachytene, the accessory chromosome in *F. arundinacea* appears to have a more symmetrical pattern than is the case in *F. pratensis.* In the latter species (BOSEMARK, 1954 a, 1956 b), one of the arms of the accessory chromosome consists mainly of a large heterochromatic segment with a small knob formation at the distal end. The other arm is more differentiated showing three





Figs. 7—9. Paired accessory chromosomes at pachytene in *F. arundinacea.* — Figs. 10, 11. Paired standard accessory chromosomes at pachytene in *F. pratensis* — Fig. 7. ×2570. — Figs. 8—11. ×4100.

separate heterochromatic blocks of which the central one is the largest. The distal end of this arm also has a characteristic knob formation. At early pachytene stages in *F. arundinacea* it is, however, difficult to differentiate between the two arms both having relatively long, weakly

staining distal segments and often only one clearly visible heterochromatic block. As the total pattern of the accessory chromosome in *F. arundinacea* is unknown, it is perhaps not justified to speculate too much about the meaning of these differences. It is quite likely, however, that the accessory chromosomes in the two species have a common origin and that a differentiation has taken place later on.

The behaviour of the accessory chromosomes in *F. arundinacea* at later stages of meiosis fully corresponds to that found for the standard type in *F. pratensis* (BOSEMARK, 1954 a). Thus, when unpaired at metaphase I, they are generally situated close to the equator. It left between the poles at anaphase I the univalents split, being probably distributed at random in the second division. In the plants with two accessory chromosomes, these were largely present as bivalents at metaphase I with normal disjunction at anaphase I. At the tetrad stage micronuclei from eliminated accessory chromosomes were seen in a low but varying frequency. The general course of meiosis in *F. arundinacea* has been very carefully studied by CROWDER (1953), and my observations are in full agreement with his. Thus, at anaphase I, sticky bridges were present in varying frequency in all plants studied although the fixations were made under favourable conditions in the experimental field.

The first pollen mitosis has been studied in three plants with accessory chromosomes. As in *F. pratensis* (BOSEMARK, 1954 a, 1956 b), the accessory chromosomes were found to undergo non-disjunction directed to the generative nucleus at anaphase of this division (Figs. 5, 6). In a few cases, it was observed that the ends of the lagging daughter chromosomes were directed towards the poles (Fig. 6), indicating a neocentric activity of the ends (BOSEMARK, 1956 b). In other respects, the first pollen mitosis appeared to be normal; and sticky bridges, as observed in plants of *F. pratensis* with high numbers of accessory chromosomes, were not observed.

3. Inheritance of the accessory chromosomes

The inheritance of the accessory chromosomes was studied in two reciprocal crosses $(42+1 \text{ acc.}) \times 42$ and $(42+2 \text{ acc.}) \times 42$. The plants were highly self-sterile and gave only a few seeds after isolation of two panicles. The chromosome numbers in the progeny plants are given in Table 3.

Although scarce, the data clearly confirm the cytological observations

TABLE 3. *Inheritance of accessory chromosomes in Festuca arundinacea.*

Parents			F_1			Total number of plants	M. acc. chr. per plant
Crosses	Plant Nos. ♀ ♂	Number of acc. chr. ♀ ♂	Number of acc. chr. 0	1	2		
1/54	11× 8	1×0	26	15	—	41	0.37
2/54	8×11	0×1	5	—	2	7	0.57
3/54	3×23	2×0	2	20	3	25	1.04
4/54	23× 3	0×2	4	27	20	51	1.31

on the directed non-disjunction of the accessory chromosome at the first pollen mitosis. As in *F. pratensis*, this mechanism is confined to the male side (BOSEMARK, 1954 b). In the cross 4/54, the non-disjunction mechanism has functioned in only 43 per cent of the cases; but with the knowledge of the variation in this respect as found in *F. pratensis*, this should not be generalized. Also with respect to the elimination of accessory chromosomes in crosses of this type, the agreement between the two species is very good.

4. Discussion

Although established population plants were not studied in *F. arundinacea*, the data obtained from the seeds collected indicate that the frequency of accessory chromosomes in the particular population must be high. As the species is quite variable in England and different native forms occupy distinct habitats, — the tall, robust forms generally grow on the heavy soils, whilst the shorter, less coarse types are found on drier calcareous and sandy soils in hills and downs grassland (HUBBARD, 1954) — a study of the frequency of accessory chromosomes in these different forms would be of considerable interest.

The question of the relationship between the accessory chromosomes in *F. arundinacea* and *F. pratensis* has been impossible to answer. However, the cytological behaviour as well as the mode of transmission of the accessory chromosomes seems to be identical in the two species. Therefore, in spite of the morphological differences observed at pachytene, one is inclined to suggest that they have a common origin. This idea is strengthened by the knowledge of the probable relationship between the two species (JENKIN, 1934; NILSSON, 1940). Hybrids between *F. arundinacea* and *F. pratensis* have been obtained in

artificial crosses by JENKIN (1934), NILSSON (1940), and MEYRS and HILL (1947), and such hybrids are probably also produced relatively often in nature (JENKIN, 1934). The F_1 hybrids are highly sterile, but occasional back-cross derivatives have been obtained by NILSSON (1940) and JENKIN (1955). Thus, it cannot be excluded that the accessory chromosome may have originated in any one of the two species after species differentiation and that it has later on been transferred to the other, It is also possible that accessory chromosomes were present in *F. pratensis* (or its progenitor) when this species took part in the origin of the hexaploid *F. arundinacea*. The lack of complete morphological correspondence between the accessory chromosomes in the two species, even if proved to be of general occurence, is of course compatible with both these possibilities.

B. Poa trivialis

Poa trivialis L. is a perennial grass, loosely tufted and with creeping, leafy stolons. It is present troughout Europe, temperate Asia and N. Africa and has been introduced into America and Australia (HUBBARD, 1954). The chromosome number $2n=14$ has been determined by, among others, AVDULOV (1931), ÅKERBERG (1942), KIELLANDER (1942), and GUINOCHET (1943). ÅKERBERG and CHRISTOFF (1942) have reported plants with $2n=15$, but it is probable that these plants were trisomic for an ordinary chromosome. GUINOCHET (1943) also found tetraploids with $2n=28$.

1. Material collected

From four natural populations in the Provinces of Skåne and Västergötland, twenty-six plants were collected altogether. Chromosome counts in root-tips showed that all plants had 14 normal chromosomes

TABLE 4. *Frequency of accessory chromosomes in spontaneous material of Poa trivialis from Sweden.*

Sample Nos.	Number of plants with respective numbers of acc. chr.					Total number of plants	% plants with acc. chr.
	0	1	2	3	4		
1/54	9	—	—	1	—	10	
7/54	8	—	—	—	—	8	
25/54	3	—	—	—	—	3	
51/54	3	—	—	1	1	5	
	23	—	—	2	1	26	11.5

but that three plants had in addition 3—4 accessory chromosomes (Table 4). The material is too small to permit any general conclusions with respect to the frequency of accessory chromosomes in natural populations of this species, but the relatively high numbers in the three plants obtained suggest that, at least in the populations where these plants were taken, accessory chromosomes are rather common.

2. Cytological observations

The size and appearence of the accessory chromosomes can be seen in Figs. 26, 27. They have a median centromere and the length is only $^1/_4$—$^1/_5$ of the average for the ordinary chromosomes. Due to the presence of large intercalary blocks of heterochromatin in the chromosomes of the normal complement, it cannot be said with certainty that the accessory chromosomes are positively heteropycnotic in resting nuclei and at early prophase in root-tips. However, this seems very likely since they have been found to be clearly heterochromatic in the meiotic prophase. It was first believed that the number of accessory chromosomes was constant in the root-tips, but as will be seen later on, a variation was found in the floral parts. Therefore a renewed study of the original root-tip slides was undertaken. One of these had unfortunately been lost, but in the other two as many metaphase plates as possible were counted in all roots present in the slide. At the same time a number of the slides from plants without accessory chromosomes were reinvestigated in the same way. To get a better idea of the inter-root variation than could be obtained from the original slides with their small number of root-tips, more extensive fixations were made from 12 young seedlings. The results of the chromosome countings are given in Table 5.

In plant 27 which was originally determined to have $2n=14+3$ acc., this number was invariably found in the 61 cells which could be studied. In plant 31 which had been considered to have $2n=14+4$ acc., this number was found in 93 cells from three root-tips; whereas in the fourth root, the 14 cells, where the chromosome number could be counted, all had $2n=14+3$ acc. This root was much smaller than the others, and the fixation was inferior and it had probably for that reason been discarded at the earlier study. It should be pointed out that, as a rule, all the original chromosome numbers were based on 5—6 cells from three different root-tips. None of the plants to which the chromosome number $2n=14$ had originally been given had cells with accessory chromosomes.

TABLE 5. *Chromosome numbers in root-tips from different plants of Poa trivialis.*

Plant Nos.	Roots	Number of cells with respective numbers of acc. chr.					Total number of cells
		0	1	2	3	4	
10	1—2	20	—	—	—	—	20
13	1—3	34	—	—	—	—	34
14	1—2	66	—	—	—	—	66
22	1—3	23	—	—	—	—	23
23	1—4	92	—	—	—	—	92
27	1—4	—	—	—	61	—	61
28	1—4	36	—	—	—	—	36
29	1—3	89	—	—	—	—	89
30	1—4	45	—	—	—	—	45
31	1—3	—	—	—	—	93	93
„	4	—	—	—	14	—	14
3/55: 1	1—6	—	—	43	—	—	43
„ : 2	1—3	—	—	—	12	—	12
„ : 3	1—10	—	—	—	72	—	72
„ : 4	1—7	—	—	43	—	—	43
„ : 5	1—5	—	—	29	—	—	29
„ : 6	1—9	—	—	68	—	—	68
„ : 7	1—9	—	—	82	—	—	82
„ : 8	1—6	—	51	—	—	—	51
„ „	7	—	—	8	—	—	8
„ „	8	8	—	—	—	—	8
„ : 9	1—8	—	—	—	65	—	65
„ : 10	1—9	—	—	65	—	—	65
„ : 11	1—10	—	—	69	—	—	69
„ : 12	1—9	—	—	67	—	—	67

In the seedlings, the chromosome numbers in the different roots from an individual corresponded in all but one plant, *viz.* 3/55: 8. Here, six roots had two accessory chromosomes, and two had one and none, respectively. It can of course not be excluded that the root with three accessory chromosomes in plant 31 was an admixture, but it is very

TABLE 6. *Variation in number of accessory chromosomes in the floral parts in two plants of Poa trivialis.*

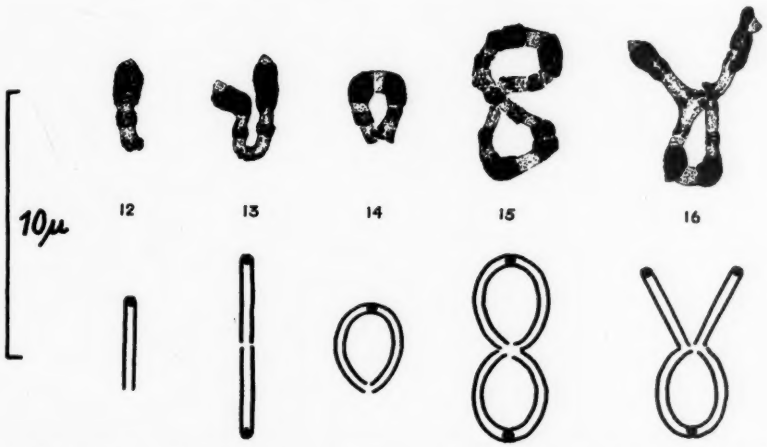
Plant	Panicle	Slide (Flower)	Number of PMC:s with respective numbers of acc. chr.						Total number of cells
			0	1	2	3	4	5	
31	I	1	—	—	—	43	34	—	77
	II	1	—	—	—	1	8	5	14
	II	5	—	1	19	3	15	2	40
	III	1	—	—	—	—	13	—	13
	III	2	—	—	—	—	24	—	24
	III	3	—	—	—	30	2	—	32
	III	4	—	—	1	48	18	—	67
	IV	2	—	—	—	—	14	—	14
	IV	3	—	—	—	—	32	—	32
	IV	5	—	—	—	55	19	—	74
	IV	6	—	—	—	24	—	—	24
	IV	7	—	—	—	—	25	—	25
	V	1	—	—	—	—	25	—	25
	V	2	—	—	—	—	19	—	19
	V	3	—	—	—	—	16	—	16
	V	5	—	—	—	—	16	—	16
	V	7	—	—	—	—	85	—	85
	V	9	—	—	—	—	51	—	51
27	I	1	—	—	—	—	44	—	44
	I	3	—	—	—	—	23	—	23
	II	1	—	—	—	—	2	32	34
	II	2	—	—	—	—	—	17	17
	II	4	—	—	—	—	—	16	16
	II	6	—	—	—	—	—	12	12
	III	1	—	—	—	46	1	—	47
	III	2	—	—	—	—	53	—	53
	III	3	—	—	—	—	10	—	10
	III	5	—	—	4	10	21	—	35
	III	6	—	—	—	6	—	—	6
	III	8	—	—	—	68	1	—	69
	III	9	—	32	—	10	—	—	42
	V	2	—	—	—	—	20	—	20
	V	6	—	—	—	—	17	—	17
	V	7	—	—	—	—	106	—	106

unlikely that this is the case with root 8 from plant 3/55: 8 as this is the only root lacking accessory chromosomes. Furthermore the 12 plants recorded here were the only ones fixed and embedded on this occasion. Thus, it must be considered proved that there is occasionally a variation in number of accessory chromosomes even in the root-system. In very young plants this inter-root variation is obviously slight. However, it cannot be excluded that in old plants it is more pronounced, and that even intra-root variation sometimes does occur.

Meiosis was studied in the three original plants with accessory chromosomes and in three plants without. In the plants without accessory chromosomes, meiosis was on a whole regular. However, univalents were present in low frequency, and in one plant bridges and fragments at anaphase I indicated heterozygosity for an inversion.

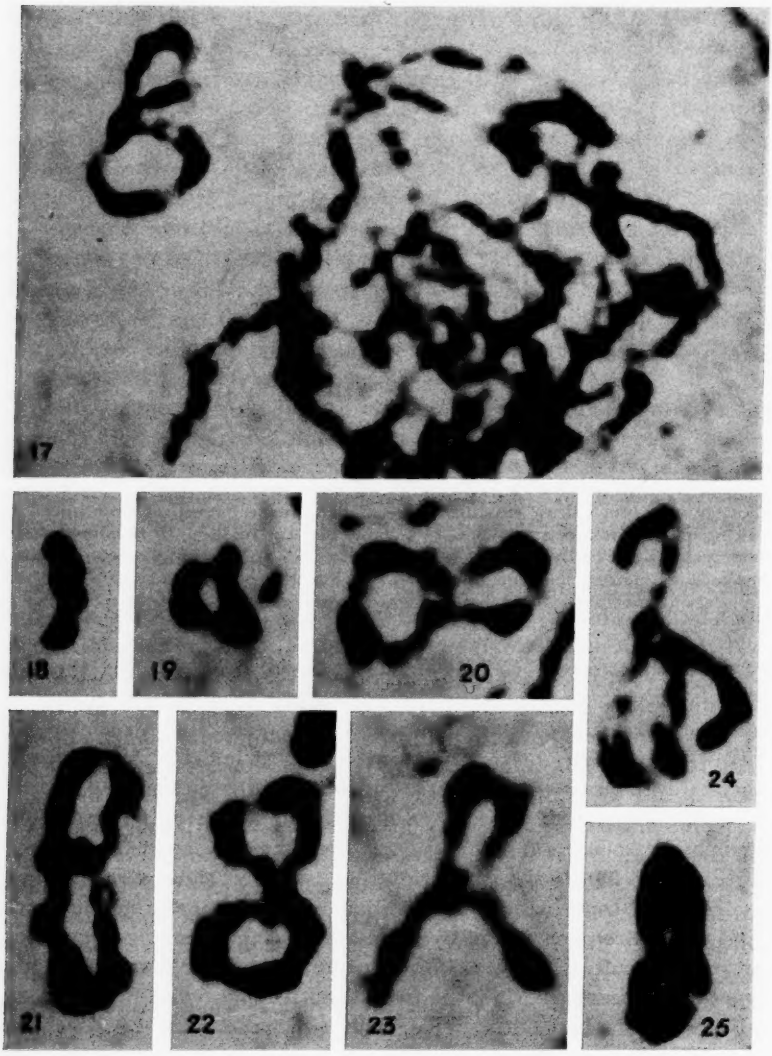
In the plants with accessory chromosomes, it was found that the number of these chromosomes was not constant in the pollen mother cells. This led to a rather careful study of this phenomenon in all three plants. The results from two of these plants are presented in Table 6. The number of accessory chromosomes was determined in different flowers from all available panicles with the appropriate stages: *viz.* diakinesis, metaphase I, and early anaphase I. The study revealed a variation not only between flowers but also within flowers and individual anthers. Generally one number is dominating in a flower, perhaps also in a panicle, but sometimes — as in plant 31, panicle II, slide 5 — there exists a wide variation. The mechanism underlying the variation is not known, but may result from loss or non-disjunction of the accessories at some stage of the early development of the panicles combined with similar events in pre-meiotic mitoses in the anthers.

The morphology and behaviour of the accessory chromosomes at pachytene could be studied in two of the plants. When univalent, the accessory chromosome seems always to pair with itself (Figs. 12, 18). Univalents were, however, relatively seldom seen at this stage, bivalents and higher configurations being most common. From the bivalents (Figs. 13, 19), it can be seen that the centromere is median, that a large heterochromatic block is present on each side of the centromere and that, in the more weakly staining distal parts of the arms, there are smaller heterochromatic blocks or associations of chromomeres. The bivalents appear to be quite symmetrical. The accessory chromosome is thus most probably an iso-chromosome. It is peculiar, however, that the ends of the bivalent always seem to be associated with each other. As a matter of fact all bivalent associations which were the result



Poa trivialis. — Figs. 12—16. Different configurations of accessory chromosomes at late pachytene together with explanatory schematic drawings. — Fig. 12. Univalent showing inter-arm pairing (fold-back). — Fig. 13. Bivalent resulting from inter-arm pairing and end to end association. — Fig. 14. Bivalent resulting from inter-chromosome pairing and end to end association. — Fig. 15. Quadrivalent association; inter-chromosome pairing and end association. — Fig. 16. Quadrivalent association due to inter-chromosome pairing between two chromosomes and inter-arm pairing in the two others; all ends associated.

of inter-chromosome pairing formed closed rings of this type. In associations of four accessory chromosomes, those most commonly observed, the accessory chromosomes were generally associated two and two through inter-chromosome pairing, but the four ends of these »bivalents» were in all cases in contact with each other (Figs. 15, 20—22). Not a single case was observed in which only three of the ends had associated and one was left free. Associations of the type seen in Figs. 16, 23, 24 with one ring and two free ends were, however, observed in some cases. The position of the heterochromatic blocks and the centromeres in the free arms showed that these configurations were the result of two accessories forming a regular bivalent through inter-chromosome pairing, the two others pairing with themselves and all four ends being associated. The schematic drawings (see Figs. 12—16) illustrate the pairing behaviour of this as well as the other types of associations found. No configurations of three accessory chromosomes were observed, but it is quite likely that such associations exist but have not been observed because most pollen mother cells studied had four accessory chromosomes.



Poa trivialis. — Figs. 17—24. Configurations of accessory chromosomes at late pachytene (see also Figs. 12—16). — Fig. 17. Quadrivalent. — Fig. 18. Univalent. — Fig. 19. Bivalent. — Figs. 20—22. Quadrivalents; inter-chromosome pairing. — Figs. 23, 24. Quadrivalents; combination of inter-chromosome and inter-arm pairing. — Fig. 25. Quadrivalent at diplotene. — ×5300.

Why the accessory chromosomes behave in the manner described, is not known. It can, however, hardly be a question of normal chiasma formation, nor is it very probable that mere stickiness is the cause of the phenomenon. It is more likely that the associations depend on some rather specific property of the end segments of the accessory chromosomes. It should be pointed out that, as far as could be judged, the chromosomes of the ordinary complement behaved in a completely normal manner.

At diplotene, the associations of four accessory chromosomes sometimes look very similar to a bivalent of two ordinary chromosomes at pro-metaphase — metaphase I (Fig. 25). The functional consequences of the associations on the behaviour at later stages of meiosis will be discussed below.

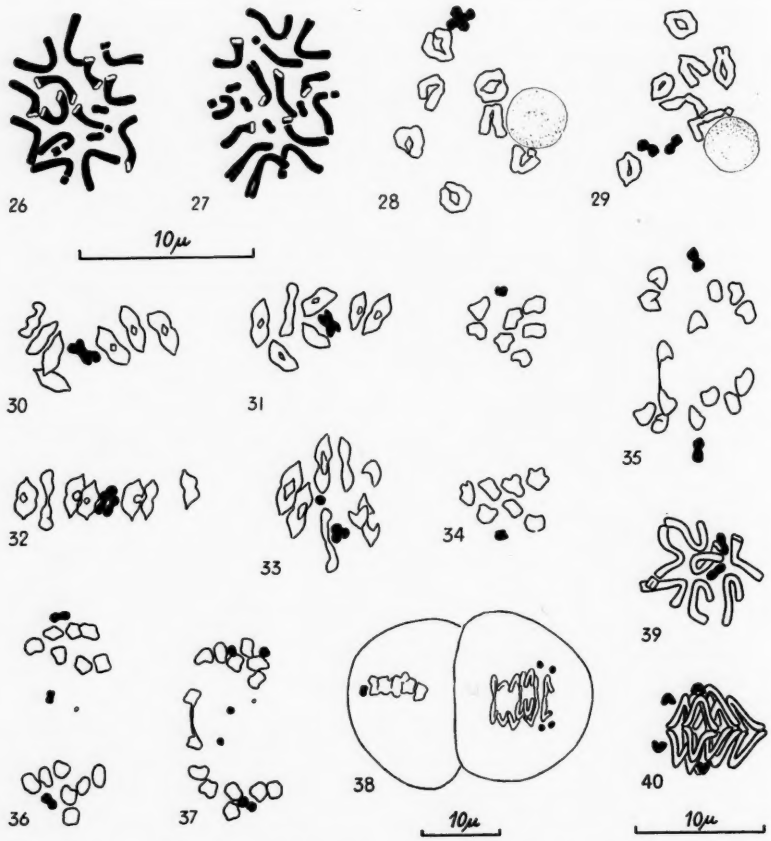
The pairing at metaphase I was studied in pollen mother cells with four accessory chromosomes from plants 27 and 31 (Table 7). In

TABLE 7. *Pairing of the accessory chromosomes in Poa trivialis at metaphase I in pollen mother cells with four accessory chromosomes.*

Plant Nos.	Configurations					Total number of cells
	1_{IV}	$1_{III}+1_{I}$	2_{II}	$1_{II}+2_{I}$	4_{I}	
27	100	4	2	—	—	106
31	72	28	39	11	1	151

plant 27, the accessories nearly always formed a quadrivalent, whereas in plant 31 more than 50 per cent of the cells showed lower configurations. At anaphase I, it was observed that when the distribution was 1—2 or 2—2 the accessory chromosomes which went to the same pole were also very often associated at this stage. At late anaphase I — telophase I, they are then similar to small bivalents (Figs. 35, 36). This most probably depends on the end to end associations just described.

Univalents left between the poles at anaphase I were observed to divide at late anaphase I — telophase I, and the daughter univalents appeared generally to arrive safely at the poles. At pro-metaphase II, the accessories largely arranged themselves in the equatorial plane and underwent a normal division at anaphase II. Univalents which have divided already at the first division are probably distributed at random at the second division. Tetrad micronuclei were very seldom observed, and the meiotic elimination appeared to be slight.



Poa trivialis. — Figs. 26, 27. Root-tip metaphase plates showing two and four accessory chromosomes respectively. — Figs. 28, 29. Diakinesis in pollen mother cells with $2n=14+4$ acc. — Figs. 30, 31, 33. Metaphase I in pollen mother cells with $2n=14+4$ acc. — Fig. 32. Metaphase I in a pollen mother cell with $2n=14+5$ acc. — Fig. 34. Anaphase I in a cell with $2n=14+2$ acc. — Fig. 35. Anaphase I in a cell with $2n=14+4$ acc. — Figs. 36, 37. Anaphase I in cells with $2n=14+5$ acc. In Fig. 37, the univalent has divided. The plant is an inversion heterozygote. — Fig. 38. Metaphase II — anaphase II. — Fig. 39, 40. The first pollen mitosis. — Fig. 39. Metaphase plate with $n=7+2$ acc. — Fig. 40. Anaphase showing normal division of the accessory chromosomes.

The first pollen mitosis was studied in two plants. It was clearly established that the accessory chromosomes disjoin normally and at the same time as the ordinary ones at anaphase (Fig. 40). Thus, in *Poa*

trivialis, there is no cytological mechanism for numerical increase of the accessory chromosomes at this stage on the male side.

3. Inheritance of the accessory chromosomes

Three crosses were made, two of which were analysed later on (Table 8). They involved plants 12 and 31 in which the chromosome numbers $2n=14+3$ acc. and $2n=14+4$ acc. had originally been counted in the root-tips. At this stage of the investigation nothing was known about the variation in number of accessory chromosomes in the panicles and therefore the parental plants were isolated together in the way previously mentioned. To save work only one root-tip from each plant was fixed, ten roots from the same number of daughter plants being embedded in the same paraffin block. For this reason the results of the crosses have a limited value. The variation between as well as within panicles will result in gametes with a range of accessory chromosomes about which we have only a restricted knowledge. Further one cannot be sure that the chromosome numbers in the roots fixed from the young seedlings always coincide with those of the zygotes although this appears largely to be the case.

In plant 12, out of a total of 164 pollen mother cells from six flowers and three panicles, 4 had one, 137 hade three, 15 had four, and 8 cells had five accessory chromosomes. Although not sufficient to be taken as a measure of the over all situation in the plant, this indicates a variation largely in the plus direction. This agrees with the result of the cross which points to a greater frequency of gametes with two accessory chromosomes than would be expected if the pollen mother cells had always contained three accessory chromosomes.

The cross 5—6/55 gave results which can very well be explained on the basis of a constant number of four accessory chromosomes together

TABLE 8. *Inheritance of accessory chromosomes in Poa trivialis.*

Parents			F_1					
Crosses	Plant Nos. ♀ ♂	Number of acc. chr. (root-tips) ♀ ♂	Number of acc. chr. 0	1	2	3	Total number of plants	M. acc. chr. per plant
1/55	12×10	3×0	—	27	41	8	76	1.75
2/55	10×12	0×3	2	26	48	15	91	1.84
5/55	31×28	4×0	1	10	56	2	69	1.86
6/55	28×31	0×4	—	20	49	3	72	1.76

with a slight degree of meiotic elimination. However, the variation in the floral parts in plant 31 seems to be almost exclusively in the minus direction. This fits in very well with the results, irrespective of the meiotic elimination.

Although the author is aware of the dangers of drawing conclusions from these crosses, there is strong indication that in *Poa trivialis* there exists no post-meiotic mechanism for numerical increase of the accessories either on the male or on the female side. Further studies are needed to explain how the variation in number of the accessory chromosomes is brought about and to find out if this variation in itself involves a mechanism of numerical increase. This would be the case if, in relation to the number of accessory chromosomes received by the zygote, the variation does result in a higher average number of accessories in the sporocytes, the gains thus being more frequent than the losses.

4. Discussion

In some respects the accessory chromosomes in *Poa trivialis* behave differently than those previously described. Thus, there is a marked numerical variation in the number of accessory chromosomes in the floral parts of an individual whereas the constancy appears to be higher in the roots. It is possible that the liability to loss and non-disjunction in the somatic cells of these organs depends on certain weaknesses in the centromere. This in turn might be connected with the fact that the accessory chromosome is very likely an iso-chromosome. However, the apparently normal and mostly central position of the accessory chromosomes in root-tip metaphase plates does not indicate any serious centromere defects. Instead it is possible that occasional failure of chromatid separation in the most proximal parts of the arms is responsible for the variation. These parts of the arms are those where the large heterochromatic blocks are situated. It may be that there is also an interaction between these segments and the centromere. There is also in *P. alpina* a slight variation in number of accessory chromosomes in the anthers, but in this species the accessories are almost entirely eliminated in the roots (MÜNTZING, 1948 c).

The end to end association between accessory chromosomes in the meiotic prophase has not been possible to explain, nor is it known if this peculiar property of the ends is connected with the heterochromatin, nor if it affects the chromosome behaviour also in somatic divisions. The lack of post-meiotic non-disjunction of the accessory chro-

mosomes makes it of special interest to find out if the variation in the panicles is mainly in the plus direction or if the accessory chromosomes in *Poa trivialis* are entirely lacking in mechanisms for a numerical increase.

C. Briza media

Briza media L. is a loosely tufted perennial grass with short rhizomes. It is relatively frequent throughout Europe, N. and W. Asia (HUBBARD, 1954). The chromosome number has been determined to $2n=14$ by, among others, KATTERMAN (1930) and AVDULOV (1931). KATTERMAN (1933, 1938 a. b) has made detailed studies of meiosis in *Briza media*, directing special attention to translocation heterozygotes found in his material. All the plants, however, had the normal chromosome number $2n=14$. In the sub-species *B. elatior* SIBTH & SM., AVDULOV (1931) found in one plant 1—3 small extra chromosomes besides the fourteen ordinary ones. The extra chromosomes were reported to have a median centromere and to vary in number within root-tips. No further study of these chromosomes was undertaken.

1. Material collected

The chromosome number was determined in a total of 58 plants from 8 different populations in southern Sweden (Table 9). Four of the plants were found to have one or two accessory chromosomes in addition to the ordinary ones. All these plants originated from one and the same population in the Province of Småland. As only seven plants were collected in this population, more than half of them had accessory chromosomes. This particular population grew on very poor moraine soil, whilst the other populations were found in old, relatively fertile meadows. However, the species grows on a wide range of soils: both light and heavy, in dry or somewhat moist conditions. In addition it has a rather wide altitudinal range. Thus, it is possible that the frequency of accessory chromosomes is in some way correlated with

TABLE 9. *Frequency of accessory chromosomes in spontaneous material of Briza media from Sweden.*

Number of plants with respective numbers of accessory chromosomes			Number of populations	Total number of plants	% plants with acc. chr.
0	1	2			
54	2	2	9	58	6.9

habitat and that more extensive collections would have yielded a higher average frequency of plants with such chromosomes.

2. Cytological observations

The appearence of the accessory chromosomes as well as of the normal complement in *Briza media* in root-tip metaphase plates is seen in Figs. 47—49. The accessory chromosome has a sub-median centromere although this is not always clearly seen in root-tip slides. There is no numerical variation of the accessory chromosomes within or between root-tips from an individual and in cases where the number was also determined in the pollen mother cells, the counts corresponded. In resting nuclei and at early prophase in root-tips the accessory chromosomes show positive heteropycnosis and are thus to be considered heterochromatic (Fig. 50).

Meiosis was studied in two plants with one and two accessory chromosomes, respectively, and in four plants without such chromosomes.

In the plants with $2n=14$, meiosis was generally normal. At diakinesis and metaphase I, most cells showed 7_{II} while 5—10 per cent of them had $6_{II}+2_{I}$ at the latter stage. No multivalents were observed. Anaphase I was also regular though, in one bivalent, the separation was sometimes a little delayed. This was noted also by KATTERMAN (1933). Later stages were normal. In one of the plants, however, giant pollen mother cells were present in low frequency. When the majority of the cells in the anther were in metaphase I or later stages and looked quite normal, these large cells contained long, only slightly spiralized chromosomes. Whether they had the diploid or the tetraploid number of chromosomes could not always be decided, but most of them seemed to be diploid. These cells were not observed to develop further, but no attempt was made to study them in detail.

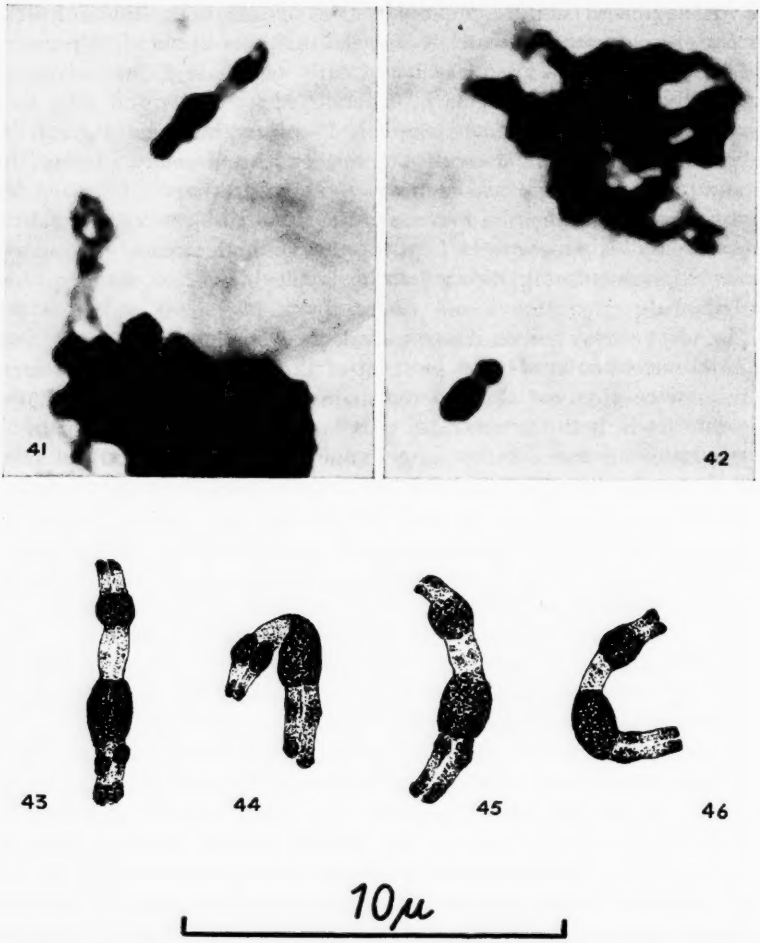
In the plant with $2n=14+1$ acc., the accessory chromosome had moved to the equatorial region in 91 of 128 cells studied at metaphase I. It was never seen to pair with members of the normal complement. Tetraploid or even hexaploid pollen mother cells were present in low frequency (about 5 %). They showed a high number of multivalents and univalents and often formed restitution nuclei at late anaphase I. Also at later stages, these cells showed various irregularities giving rise to giant pollen tetrads, frequently with persisting bridges and micronuclei. Large pollen mother cells with unspiralized chromosomes, as described previously, were also present in this plant and here some of them evidently had an increased number of chromosomes.

At anaphase I in the diploid pollen mother cells, the univalent accessory chromosome was left between the poles in about 90 per cent of the cells. At late anaphase I and early telophase I, the univalents generally divided. Univalents in different stages of division were seen in low frequency also at late telophase I — interphase. As is seen from Figs. 55—57, dividing univalent accessory chromosomes display the same type of terminal neo-centric activity as previously described for accessory chromosomes in *Festuca pratensis* (BOSEMARK, 1956 b). Thus, the chromatids appear to be kept together on both sides of the centromere, the ends being directed towards the poles and more or less attenuated.

In most cases, the daughter univalents were included in the telophase nuclei but in about 5 per cent of the cells at interphase micronuclei were observed close to the main nuclei. At metaphase II, the accessories in both the daughter cells had moved to the equator in 66 per cent of the cases. In 26 per cent one of the daughter cells showed an accessory chromosome at one of the poles, while in 8 per cent both the accessory chromosomes were outside the plate. At anaphase II, a lagging accessory chromosome was seen in one or both of the daughter cells in 65 per cent of the cases. At the tetrad stage, however, only 20 per cent of the tetrads had a micronucleus in one of the cells. Tetrads with micronuclei in two cells were very rarely seen. As the micronuclei were heavily stained and easy to observe, this suggests that in spite of the frequent splitting of univalents at anaphase I the meiotic elimination is relatively moderate.

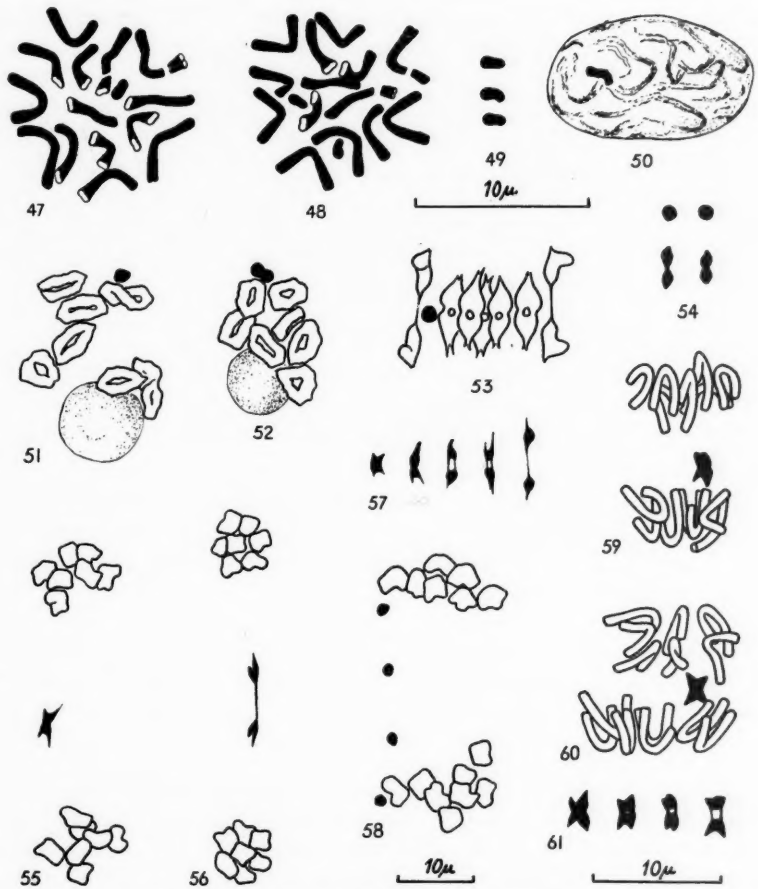
In plant 51 with two accessory chromosomes, these could be studied at the meiotic prophase. However, only at late pachytene were a number of cells obtained where the accessory chromosomes were lying free in the cytoplasm (Figs. 41, 43—46). Although more detailed studies of the morphology of the accessory chromosomes could consequently not be performed, it was confirmed that the centromere has a sub-median position and that both arms have large heterochromatic segments. In the long arm, a second smaller heterochromatic segment can also be seen at the distal side of the large one. The distal portions of the arms appear to be largely euchromatic, but they both end with a small knob or pair of large chromomeres. The heterochromaty is also clearly manifested at diplotene and diakinesis (Fig. 42).

At metaphase I in the same plant, the normal bivalents were very crowded. Therefore, when the accessory chromosomes were situated on the equator it could not always be decided whether they were paired, but



Briza media. — Figs. 41—46. Paired accessory chromosomes at the meiotic prophase. — Figs. 41, 43—46. Late pachytene. — Fig. 42. Diplotene. — Figs. 41, 42. ×4230.

probably they were, in most cases. In 8 of 57 cells, one accessory chromosome was off the plate while in 9 cells both univalents were at the poles. Anaphase I seemed to be fully normal, but too few cells were obtained to permit reliable observations. At metaphase II, the accessory chromosomes were situated on the equator in most cells. At ana-



Briza media. — Figs. 47, 48. Root-tip metaphase plates. — Fig. 47. $2n=14+1$ acc. — Fig. 48. $2n=14+2$ acc. — Fig. 49. Accessory chromosomes, separately drawn. — Fig. 50. Prophase in a plant with $2n=14+1$ acc. showing positive heteropycnosis of the accessory chromosome. — Figs. 51—58. Meiosis in plants with one and two acc. chr. — Fig. 51. Diakinesis in a plant with $2n=14+1$ acc. — Fig. 52. The same in a plant with $2n=14+2$ acc. — Fig. 53. Metaphase I in a plant with $2n=14+1$ acc. — Fig. 54. Univalents and bivalents of acc. chr., separately drawn. — Figs 55, 56. Anaphase I in plants with $2n=14+1$ acc. showing dividing univalent acc. chr. — Fig. 57. Dividing univalent acc. chr., separately drawn. Note that the ends of the chromatids are drawn out and directed towards the poles. — Fig. 58. Anaphase I in a plant with $2n=14+2$ acc. Univalent acc. chr. have divided. — Figs. 59—61. The first pollen mitosis in plants with $2n=14+1$ acc. — Figs. 59, 60. Anaphase showing non-disjunction of the accessories. — Fig. 61. Configurations of acc. chr. at anaphase, separately drawn.

phase II, lagging accessory chromosomes were rather seldom seen. In 5 per cent of the tetrads observed, one of the cells had a micronucleus.

The first pollen mitosis was studied in two plants with accessory chromosomes. The accessory chromosome was found regularly to undergo non-disjunction directed towards the generative nucleus at anaphase of this division (Figs. 59, 60). As in previous cases, the two chromatids are held together on each side of the centromere. Very often the free ends were drawn out and directed towards the poles (Figs. 59—61). In other respects the first pollen mitosis, as well as later stages of pollen development, appeared to be fully normal.

3. Inheritance of the accessory chromosomes

Two crosses of the type (14+2 acc.)×14 and reciprocal were made in the usual way. Unfortunately, in one of the combinations one of the parental plants died during flowering, and the other consequently set few seeds. (Table 10).

TABLE 10. *Inheritance of accessory chromosomes in Briza media.*

Parents			F_1					Total number of plants	M. acc. chr. per plant
Crosses	Plant Nos. ♀ ♂	Number of acc. chr. ♀ ♂	Number of acc. chr. 0	1	2	3	4		
1/55	51× 7	2×0	1	60	1	—	—	62	1.00
2/55	7×51	0×2	11	11	30	1	3	56	1.54
3/55	55×62	2×0	3	9	2	—	—	14	0.93

The result of the reciprocal cross shows that the mechanism of non-disjunction of the accessory chromosomes in the pollen grains has functioned in about 75 per cent of the cases but that there is no such mechanism on the female side. Also the sparse data from cross 3/55 are in line with this. Besides the plants listed in the table, two trisomic plants with one and two accessory chromosomes, respectively, one triploid with two accessories and one diploid with a telocentric chromosome were obtained in cross 1/55. The telocentric chromosome had probably originated through misdivision in the centromere of an ordinary chromosome. Although no tetraploid pollen mother cells were observed in plant 51, the triploid progeny plant shows that — at least on the female side — such sporocytes are occasionally produced. The elimination of accessory chromosomes in the present crosses is in agreement with the cytological observations.

4. Discussion

The most interesting observation in *Briza media* is the demonstration of still another case of non-disjunction of accessory chromosomes at the first pollen mitosis. In this, as in practically every other respect, the study has revealed nearly complete correspondence between the behaviour of the accessory chromosomes in *B. media*, and that in *Festuca pratensis* and *Phleum phleoides* (BOSEMARK, 1954 a and b, 1956 c). The material is very small; but if we allow for the same amount of inter-plant variation as has been found in the two last mentioned species, a generalization of the present observations on the accessory chromosomes in *B. media* seems warranted. It cannot be excluded, however, that also other types of accessory chromosomes exist in *B. media.*

As accessory chromosomes obviously are present in *B. elatior* as well (AVDULOV, 1931), it is quite possible that such chromosomes occur also in the annual species *B. minor* and *B. maxima.* If so, these would be more favourable than the perennials for studies of changes in frequency of accessory chromosomes in natural and synthetic populations under different environmental conditions.

D. Holcus lanatus

Holcus lanatus L. is a perennial occurring in most of Europe, temperate Asia and N. W. Africa; it has been introduced into N. America and other temperate parts of the world (HUBBARD, 1954). The chromosome number $2n=14$ has been determined by AVDULOV (1931), LITARDIÈRE (1949), and BEDDOWS and JONES (1953). None of these authors have mentioned the occurrence of accessory chromosomes in their materials.

1. Material collected

The material was collected in natural populations in the Provinces of Skåne and Västergötland and consisted of 73 plants from 9 localities. The chromosome numbers are given in Table 11.

As may be seen, all but one sample contained some plants which had accessory chromosomes. Although the frequency of such plants is relatively low and all but one have only a single accessory chromosome, the data indicate that accessory chromosomes are present in a varying frequency in most populations of *Holcus lanatus* in southern Sweden.

TABLE 11. *Frequency of accessory chromosomes in spontaneous material of Holcus lanatus from Sweden.*

Sample Nos.	Number of plants with respective numbers of acc. chr. 0	1	2	Total number of plants	% plants with acc. chr.
1/54	11	1	1	13	
6/54	6	—	—	6	
7/54	4	—	—	4	
12/54	5	2	—	7	
18/54	7	2	—	9	
20/54	7	2	—	9	
21/54	9	1	—	10	
22/54	5	1	—	6	
30/54	6	3	—	9	
Total	60	12	1	73	17.8

2. Cytological observations

The length of the accessory chromosomes in root-tip metaphase plates is about $^1/_3$ of the average for the ordinary chromosomes (Figs. 74, 75). With the type of fixative used, the position of the primary constriction is not always seen in root-tip cells. Observations at pachytene (Figs. 62—65, 68—73) and at the first pollen mitosis (Figs. 90, 91), however, have shown it to be sub-median. In resting nuclei, the accessory chromosomes can be seen as darkly staining bodies and must be considered largely heterochromatic (Fig. 76). The number of accessory chromosomes in the root-tips was found to be constant for an individual. Where counts were made in anthers, the number always corresponded with that in the roots.

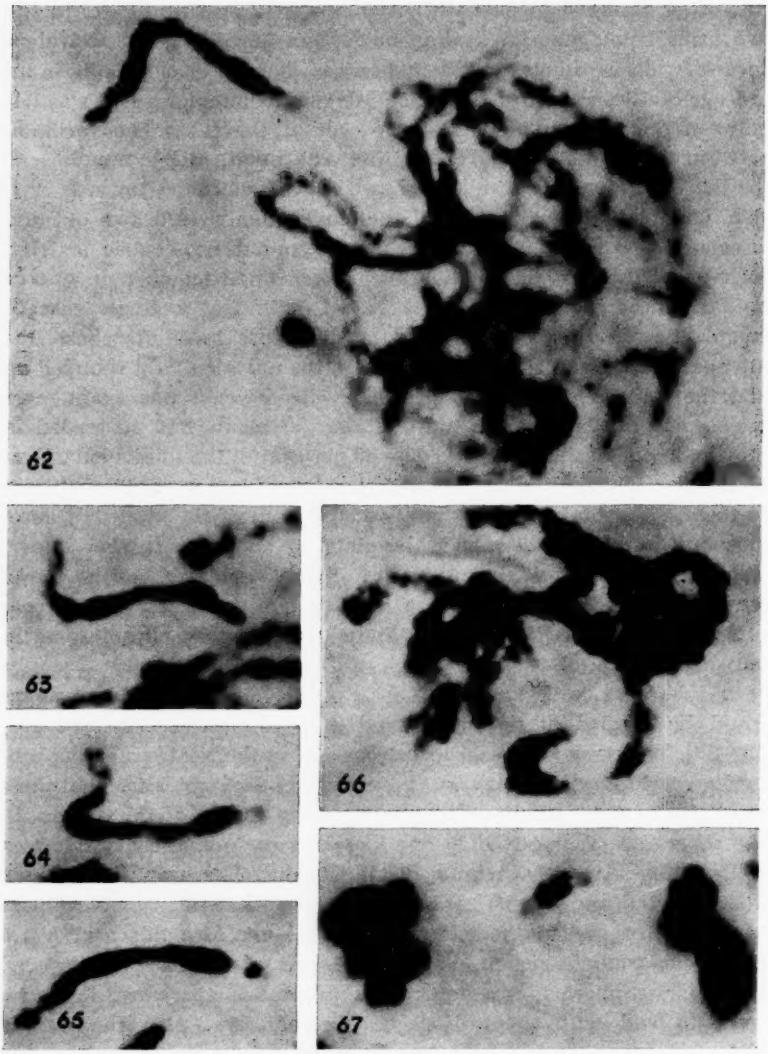
Meiosis has been studied in eight plants: two without, five with one, and one with two accessory chromosomes. In the plants without accessory chromosomes, meiosis was regular with 7_{II} at metaphase I. Univalents were seldom seen, and anaphase I also was normal. In three of the five plants with one accessory chromosome, a low frequency of bridges and fragments were observed at anaphase I, indicating heterozygosity for an inversion. In the other two plants the behaviour of the ordinary chromosomes was on a whole regular. The three first mentioned plants were all daughter plants originating from the cross 1/55 (Table 12), and the parental plants were thus probably structurally different.

At pachytene in the plants with one accessory chromosome, this

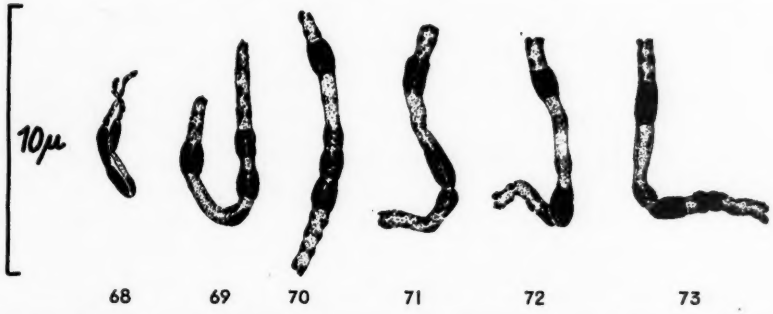
was often observed to pair with itself, heterochromatic blocks in the two arms being associated (Fig. 68). At metaphase I, the univalent accessory chromosome was situated on the equator, or close to it, in an average of 70 per cent of the cells observed. At anaphase I, it was left between the poles in about 80 per cent of the cells. The variation between the plants in this respect was very small. At late anaphase I, the lagging univalents regularly showed clear tendencies towards division. One or both of the ends of the chromatids were drawn out and directed towards the poles, indicating terminal neo-centric activity. In spite of this, the chromatids always remained together on one or both sides of the centromere (Figs. 84—86). Not a single case of complete separation was observed. At telophase I — interphase, the attenuated ends had largely disappeared, the univalent had rounded up and the staining, which had previously been weak, was again more intense. At the latter stage most of the univalents had succeeded in moving to one of the poles, and 75—80 per cent of the undivided univalents had either been included in the main nuclei or were situated close to them. At metaphase II, only 10—15 per cent of the accessory chromosomes were situated outside the plate. At anaphase II, they underwent a normal division, and laggards were very rare. At the tetrad stage, micronuclei were likewise very seldom seen: less than 5 per cent of the tetrads showed an eliminated accessory chromosome in one of the four cells.

In the plant with $2n=14+2$ acc., paired accessory chromosomes could be studied at pachytene and early diplotene (Figs. 62—65, 69—73). The centromere is evidently located in the weakly staining submedian region, and in some cases one also got the impression that there were two or more small chromomeres in the middle of this region. In the short arm, generally only one large heterochromatic block is seen followed by a weakly staining distal segment. The long arm is more differentiated and often shows three separate heterochromatic blocks of which the one in the middle is the largest. Also this arm has a distal euchromatic region. In these regions of both arms, however, larger or smaller chromomeres can be seen; furthermore, the arms seem to end with pairs of large chromomeres. Also the normal chromosomes were observed to possess heterochromatic regions, but both absolutely and proportionally the accessory chromosome is by far the most heterochromatic one.

In this plant there was a low frequency of univalents from the normal complement, but otherwise meiosis was regular. At diakinesis,



Holcus lanatus. — Figs. 62—65. Paired accessory chromosomes at pachytene. — Fig. 66. Paired acc. chr. at diplotene. — Fig. 67. Univalent acc. chr. at late anaphase I showing tendency to division. — Figs. 62—66. ×4000. — Fig. 67. ×2400.



Holcus lanatus. — Figs. 68—73. Accessory chromosomes at pachytene, separately drawn. — Fig. 68. Fold-back of univalent acc. chr. — Figs. 69—73. Bivalents.

the accessory chromosomes were practically always associated. This also was the case in 75 per cent of the cells at metaphase I (Figs. 79, 80). At anaphase I, 20 per cent of the cells showed one lagging univalent and 10 per cent two. At metaphase II most accessory chromosomes had moved to the equator, resulting in normal division at anaphase II. Tetrad micronuclei were almost never seen in this plant. The accessory chromosomes never were observed to pair with the ordinary ones.

The first pollen mitosis was studied in three plants, and also in this species the accessory chromosomes were found to undergo directed non-disjunction (Figs. 90—92). The mechanism is the same as previously described. A tendency towards neo-centric activity of the chromosome ends was observed at this stage (Fig. 92). No disturbances of the first pollen mitosis were observed which could be related to the presence of accessory chromosomes. The further development of the pollen grains seemed to be fully normal.

3. Inheritance of the accessory chromosomes

To study the transmission of the accessory chromosomes, crosses were made in the usual way by isolating the parental plants. The chromosome numbers of the progeny plants are given in Table 12.

The results of the crosses verify the cytological observations on the non-disjunction of the accessory chromosomes in the pollen grains. There appears to be no such mechanism on the female side. In cross 1/55 the three plants with more than one accessory chromosome may be explained by the fact that plant 56 proved to be slightly self-fertile.

TABLE 12. *Inheritance of accessory chromosomes in Holcus lanatus.*

Parents				F_1					
Crosses	Plant Nos. ♀ ♂	Number of acc. chr. ♀ ♂	Number of acc. chr. 0	1	2	3	Total number of plants	M. acc. chr. per plant
1/55	56×54	1×0	12	7	2	1	22	0.64
2/55	54×56	0×1	46	5	20	—	71	0.63
3/55	48×39	1×0	39	26	—	—	65	0.40
4/55	39×48	0×1	59	15	15	—	89	0.51
7/55	1×61	2×0	1	8	—	—	9	0.89
8/55	61× 1	0×2	—	1	4	—	5	1.80

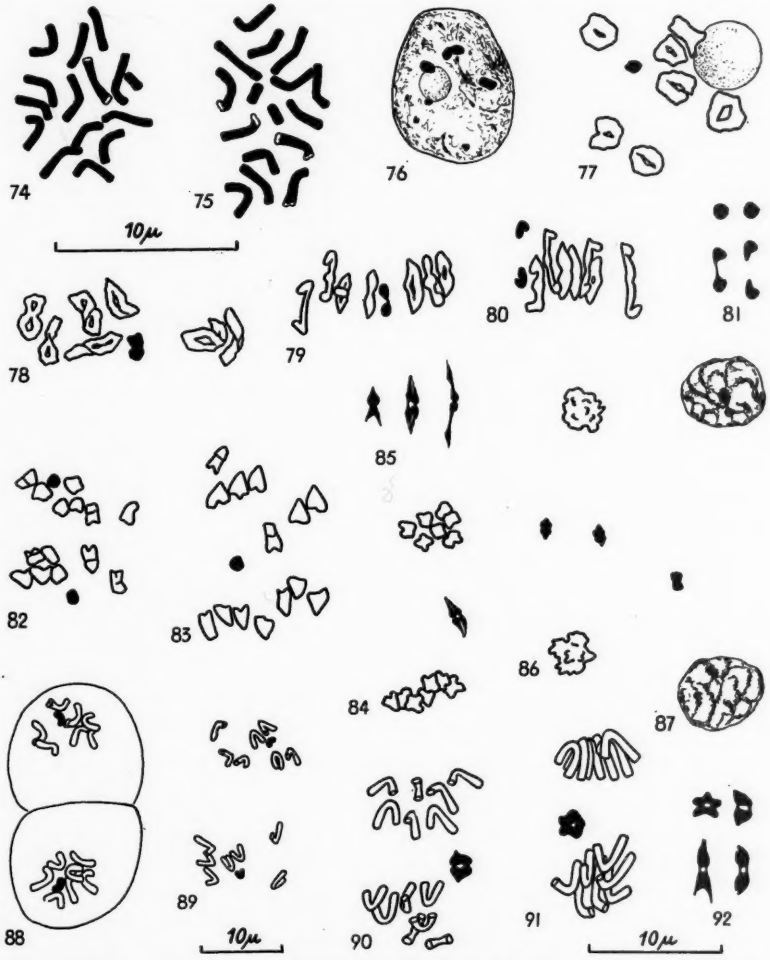
The elimination of univalent accessory chromosomes amounts to 20—30 per cent. This is not a very high figure, but still too high to agree with the scarcity of tetrad micronuclei. The reason for this discrepancy is not known but may be due to the fact that micronuclei are sometimes resorbed in the cytoplasm at an earlier stage. In the cross involving the plant with two accessory chromosomes, the number of progeny plants is, unfortunately, very small, but nevertheless the results are in conformity with expectation.

The general effectiveness of the mechanism of non-disjunction cannot be judged from these few crosses. In conformity with observations in other materials, there is quite likely a variation between plants in this respect.

4. Discussion

The result of the collections suggests that plants with accessory chromosomes are present in low frequency in most populations of *Holcus lanatus* in southern Sweden. The consistant low frequency of such plants and the fact that only a single plant had more than one accessory chromosome, in spite of the non-disjunction mechanism, may have depended upon selection against plants with accessory chromosomes in the populations sampled. Even if this be the case, it is quite possible that in other genotypes, or under other environmental conditions, the frequency is considerably higher.

In *Holcus lanatus* univalent accessory chromosomes lagging between the poles at anaphase I were never observed to divide. In this respect, they resemble the accessory chromosomes in *Anthoxanthum aristatum* (ÖSTERGREN, 1947) and *Poa alpina* (MÜNTZING, 1948). Otherwise the cyto-



Holcus lanatus. — Figs. 74—75. Root-tip metaphase plates. — Fig. 74. $2n=14+1$ acc. — Fig. 75. $2n=14+2$ acc. — Fig. 76. Resting nucleus in root-tip from a plant with $2n=14+3$ acc., showing positive heteropycnosis of acc. chr. — Figs. 77—89. Meiosis in plants with one and two acc. chr. — Fig. 77. Diakinesis in a plant with $2n=14+1$ acc. — Fig. 78. The same from a plant with $2n=14+2$ acc. — Figs. 79, 80. Metaphase I in a plant with $2n=14+2$ acc. — Fig. 81. Univalents and bivalents of acc. chr., separately drawn. — Fig. 82. Anaphase I in a plant with $2n=14+2$ acc. — Fig. 83. The same from a plant with $2n=14+1$ acc. — Fig. 84. Late anaphase I from the same plant with the univalent showing tendency to division. — Fig. 85. Lagging univalent acc. chr., separately drawn. Note that the chromatids are drawn

logical behaviour, as well as the transmission of accessory chromosomes, is similar to that of the previous species. *Holcus lanatus* thus furnishes yet another example of an accessory chromosome with a non-disjunction mechanism.

E. Alopecurus pratensis

Alopecurus pratensis L. is a tufted perennial grass, widespread in Europe and N. Asia. It has been introduced into N. America and elsewhere (HUBBARD, 1954). Several workers have determined the chromosome number to $2n=28$. Meiosis has been studied by, among others, CHURCH (1929), RANCKEN (1934), JOHNSON (1941), and by WÖHRMANN (1955). The latter three workers all found some plants, which in addition to the ordinary 28 chromosomes, had one or two small chromosomes. These were all of the same type and evidently belonged to the category of accessory chromosomes. As the accessory chromosomes in *A. pratensis* had not been subjected to a closer investigation, this species was included in the present study.

1. Material collected

The material originated from natural populations in the Provinces of Skåne, Västergötland and Småland. The chromosome number was determined in 70 plants from 9 different populations (Table 13).

As may be seen, about 40 per cent of the plants, besides the ordinary 28 chromosomes, also had 1—6 accessory chromosomes. Only two of the samples were lacking in such plants. As the total number of plants per locality is small, plants with accessory chromosomes are obviously very common in this species in southern Sweden.

2. Cytological observations

In root-tip metaphase plates the appearence of the accessory chromosomes in my material fully corresponds with that described by RANCKEN (1934). The length is $^1/_2$—$^1/_3$ of that of the normal chromosomes and the position of the centromere is sub-median—sub-terminal

out and directed towards the poles. — Fig. 86. Telophase I in a plant with $2n=14+2$ acc.; the univalent acc. chr. is still undivided. — Fig. 87. Interphase after the first division in a plant with $2n=14+1$ acc.; the univalent has not succeeded in dividing. — Fig. 88. Metaphase II in a plant with $2n=14+2$ acc. — Fig. 89. Anaphase II from the same plant. — Figs. 90, 91. Anaphase of the first pollen mitosis showing non-disjunction of accessory chromosomes. — Fig. 92. Configurations of acc. chr., separately drawn. Note that the ends are sometimes drawn out and directed towards the poles.

TABLE 13. *Frequency of accessory chromosomes in spontaneous material of Alopecurus pratensis from Sweden.*

Sample Nos.	Number of plants with respective numbers of acc. chr.							Total number of plants	% plants with acc. chr.
	0	1	2	3	4	5	6		
1/54	7	1	—	—	—	—	—	8	
7/54	11	—	—	—	—	—	—	11	
19/54	4	3	1	—	—	—	—	8	
24/54	4	2	1	2	—	—	1	10	
25/54	3	1	1	1	—	—	—	6	
27/54	2	1	3	—	—	—	—	6	
45/54	5	—	—	—	—	—	—	5	
56/54	6	—	1	—	—	—	—	7	
61/54	2	3	—	2	2	—	—	9	
Total	44	11	7	5	2	—	1	70	37.1

(Figs. 100—103). In a plant with two accessory chromosomes, however, one of these had lost more than half of the long arm, most probably through a deficiency (Fig. 101). Also in *A. pratensis*, the accessory chromosomes are positively heterochromatic in resting nuclei and at early prophase in root-tips (Fig. 104). For what is evidently the same type of accessory chromosome, WÖHRMANN (1955) reports a median centromere and that it is entirely euchromatic. His studies, however, were restricted to later stages of meiosis during which it is more difficult to decide on these matters. The number of accessory chromosomes was found to be constant for an individual plant, and the same number was also present in the anthers. This was also the case in the material studied by RANCKEN (1934).

Meiosis was investigated in 12 plants with the following numbers of accessory chromosomes:

	Number of accessory chromosomes					Total
	0	1	2	3	4	
Number of plants	3	3	3*	1	2	12

* Includes the plant with the small accessory chromosome previously mentioned.

A few remarks will first be made on the behaviour of the ordinary chromosomes. RANCKEN (1934) found strict bivalent pairing in all plants except one. This plant had two accessory chromosomes. Rings or chains were present in 9 per cent of the pollen mother cells. JOHNSON (1941) similarly found 3.5 and 1.4 per cent of quadrivalents, respec-

tively, in the two plants studied by him. Both these plants had bridges and fragments at anaphase I and one accessory chromosome each. For this reason, JOHNSON and RANCKEN associate the meiotic irregularities observed with the presence of accessory chromosomes. WÖHRMANN (1955), studying meiosis in untreated and X-rayed material of *A. pratensis*, found a low frequency of bridges but no multivalents in the untreated material. Among the plants studied by the present author only one was found where neither multivalents nor bridges and fragments were observed in varying frequency. Most plants showed, besides univalents, both these irregularities. In some plants, the frequency was estimated (Table 14).

TABLE 14. *Frequency of meiotic irregularities in five plants of Alopecurus pratensis.*

Plant Nos.	% PMC:s with 1—4 univalents	% PMC:s with multivalents	% PMC:s with bridges and fragments at A I
25	0 (129)	0 (100)	5 (136)
31	11 (80)	50 (~100)	11 (89)
36	5 (81)	7 (81)	21 (14)
41	9 (75)	2 (75)	7 (99)
43	30 (78)	5 (78)	69 (16)

Figures in brackets give number of cells studied.

The fact that bridges and fragments seem to be present in most individuals indicates that inversions are common in *A. pratensis*. The low frequency of multivalents in most plants might be due to remnants of an earlier, more pronounced partial homology between the two sets of chromosomes. Plant 31 is, however, probably heterozygous for a reciprocal translocation. There is certainly no direct relationship between the meiotic irregularities mentioned and the presence of accessory chromosomes.

At diakinesis in the plants with one accessory chromosome, this was always present as a univalent, and no clear case of pairing with members of the normal complement was observed. WÖHRMANN (1955) has photographed one cell which he suggests shows pairing between an accessory chromosome and a normal bivalent, and RANCKEN (1934) discussed the possibility of the »fragments» taking part in the chains and rings observed. Both these conclusions are very likely erroneous. In the first case, only one cell was observed. Besides the possibility that

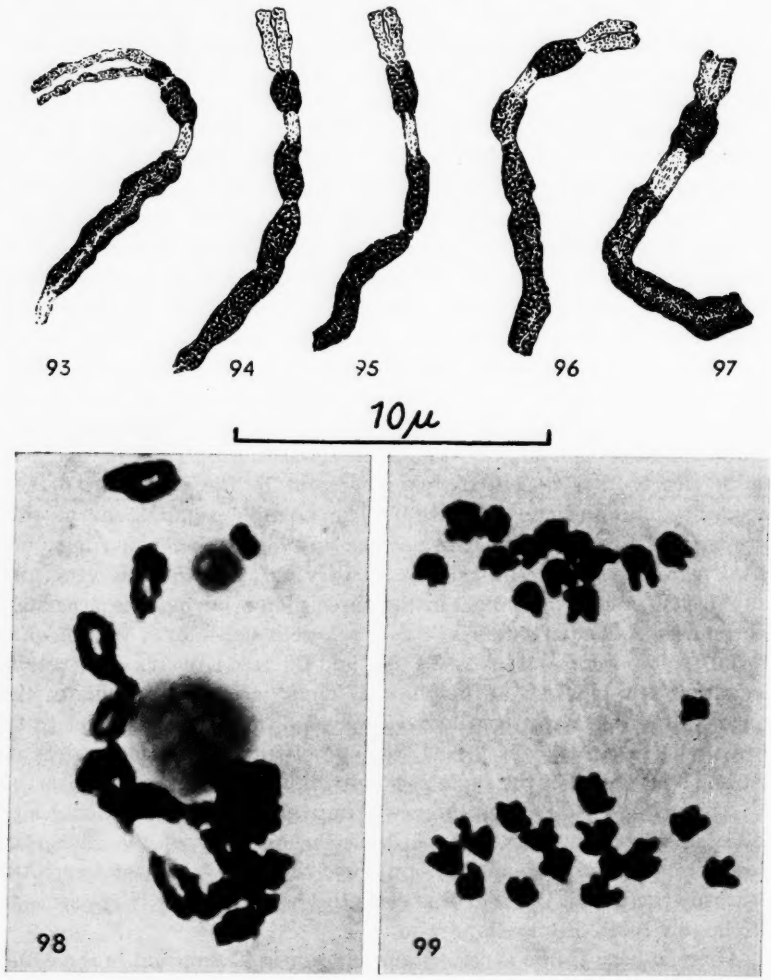
the accessory chromosome by chance had come to be situated very close to the bivalent when the cell was squashed, the heterochromaty of the accessory chromosome can occasionally make them stick to other chromosomes.

In *A. pratensis*, two pairs of the ordinary chromosomes are capable of forming nucleoli (Fig. 98). Due to fusion of these, there is, however, often only one large nucleolus present in each pollen mother cell. In the plants with accessory chromosomes, it was found that these were very often in contact with the main nucleolus at diplotene and diakinesis. When the accessory chromosome was lying free in the cytoplasm at a distance from the ordinary nucleoli, it had always formed a small nucleolus of its own (Figs. 98, 107). As the accessory chromosome must evidently have a nucleolus organizer, one might expect to find a SAT-constriction in this chromosome in somatic metaphase plates. Although the type of fixative used (chrome-acetic-formalin) is unsuitable for chromosome morphological studies, in some root-tip cells it was seen quite clearly that a constriction is present in the long arm of the accessory chromosome (Fig. 103). The possible significance of this property of the accessory chromosome will be touched upon later on.

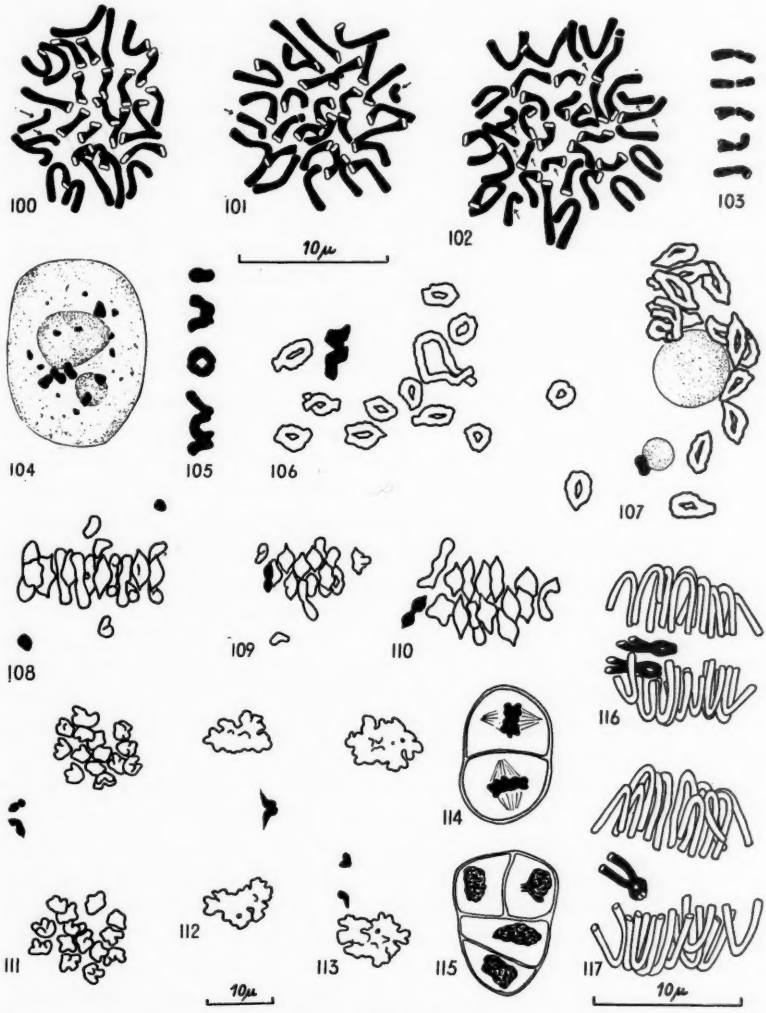
At metaphase I, the univalent accessory chromosome was generally situated at one of the poles. In the three plants having one accessory chromosome, the percentages of cells where it had moved to the equatorial region were as low as 23, 24, and 33 respectively. This results in rather few laggards at anaphase I. Thus, in one of the plants, the accessory chromosome was found undivided at one of the poles in 60 cells, whereas in 12 cells it was lagging between the anaphase groups. At late anaphase I in the same plant, dividing univalents were observed in 23 cells of 136. The appearence of univalents at different stages of division is seen in Figs. 111—113. In the majority of the cases, the long arms of the chromatids were somewhat drawn out and directed towards the poles. Thus, in this case, the neo-centric activity seems to be largely limited to the long arm.

At metaphase II, the accessory chromosomes had moved to the equator in well above 90 per cent of the cases observed. At anaphase II, laggards were relatively rare. The percentage of tetrads with a micronucleus in one or two cells ranged from 2—17.

The plants with two accessory chromosomes were rather carefully studied at pachytene. At early stages of contraction, the difference in length of the arms of the paired accessory chromosomes is not very marked (Fig. 93). The longer arm, however, is almost entirely hetero-



Alopecurus pratensis. — Figs. 93—97. Paired accessory chromosomes at pachytene of meiosis. — Fig. 98. Diakinesis in a plant with $2n=28+1$ acc. The acc. chr. has formed a nucleolus of its own. — Fig. 99. Anaphase I from a plant with $2n=28+1$ acc. The lagging univalent acc. chr. prepares to divide and the ends of the long arms are slightly drawn out already at this stage. — Fig. 98. ×1800. — Fig. 99. ×1750.



Alopecurus pratensis. — Fig. 100—102. Root-tip metaphase plates. — Fig. 100. $2n=28+2$ acc. — Fig. 101. $2n=28+2$ acc. One of the acc. chr. has lost part of the long arm probably through a terminal deficiency. — Fig. 102. $2n=28+8$ acc. — Fig. 103. Acc. chr., separately drawn. In the long arm a secondary constriction can be seen. — Fig. 104. Resting nucleus from a plant with $2n=28+6$ acc. showing positive heteropycnosis of the acc. chr. — Figs. 105—115. Meiosis in plants with various numbers of acc. chr. — Fig. 105. Different configurations of acc. chr. at diakinesis; a univalent, two bivalents, and a quadrivalent. — Fig. 106. Diakinesis in a plant

chromatic and has only a short euchromatic distal part. The short arm can be seen to consist of one, sometimes two smaller heterochromatic blocks and a long euchromatic segment. At later stages of contraction, this segment gradually decreases in size, and the centromere attains a clearly asymmetrical position (Figs. 94—97). In the plants with only one accessory chromosome, this was often observed to pair with itself.

At diakinesis in plants with two accessories, these were nearly always paired. At metaphase I, univalents were observed in about 15 per cent of the sporocytes. At anaphase I, laggards were rare, and the behaviour of the accessory chromosomes at the following stages was consequently largely normal.

Plant 31, with three accessory chromosomes, was the one previously mentioned, in which about 50 per cent of the pollen mother cells showed multivalents. The pairing of the accessory chromosomes at diakinesis and metaphase I was generally $1_{II}+1_{I}$. The distribution at anaphase I was mostly 2 — 1, but there were lagging accessory chromosomes in about 30 per cent of the cells. With respect to the accessory chromosomes, nothing is to be reported for the following stages.

However, the second meiotic spindles were often in axes at various angles to each other, and this resulted in pollen tetrads of different appearance (Figs. 114, 115). The situation was further complicated by a rather frequent occurrence of bridges. At the tetrad stage, one or two of the four cells often showed various degrees of degeneration. This was probably a consequence of the structural heterozygosity of this plant.

In the plant with four accessory chromosomes, these were all associated in 27 cells observed at diakinesis, whereas 54 cells had two bivalents (Fig. 106). At metaphase I, only the frequency of univalent accessory chromosomes outside the equatorial region was registered. Of 127 cells, 27 showed one or more univalents at the poles. Also in

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with $2n=28+4$ acc. — Fig. 107. Diakinesis in a plant with $2n=28+1$ acc. The accessory chromosome has formed a nucleolus of its own. — Fig. 108. Metaphase I in the plant with two acc. chr. one of which has lost part of the long arm. The acc. chr. are unpaired. — Fig. 109. From the same plant. The acc. chr. form a bivalent. — Fig. 110. Metaphase I in a plant with $2n=28+2$ acc. — Fig. 111. Anaphase I in a plant with $2n=28+1$ acc. The univalent has divided. — Figs. 112, 113. Telophase I from the same plant showing univalents in different stages of division. — Fig. 114. Metaphase I with disturbed spindle orientation. — Fig. 115. Pollen tetrad resulting from disturbed spindle orientation as in Fig. 114. — Figs. 116, 117. Anaphase of the first pollen mitosis showing non-disjunction of acc. chr.

this plant, bridges were frequent and spindle orientation at metaphase II — anaphase II often was abnormal.

In the plant with one accessory chromosome of the »standard» type and one with a supposed terminal deficiency in the long arm, pairing occurred between the two types in 5 cells out of 49 at diakinesis, and in 1 of 73 at metaphase I. The low incidence of bivalent formation might have depended upon, besides the loss of a portion of the long arm, some other additional structural change in the small accessory chromosome.

The first pollen mitosis was studied in a few plants with a varying number of accessory chromosomes. As seen from Figs. 116, 117, the accessory chromosomes in *A. pratensis* undergo the usual type of non-disjunction at anaphase of this division. The chromatids are held together at symmetrically placed segments on each side of the centromere, thus leaving the distal parts of the long arms free. The figure is the same as for the standard types in rye (MÜNTZING, 1946) and *Phleum phleoides* (BOSEMARK, 1956 c). Some cases were observed in which the chromatids would probably have disjoined, and in a few pollen grains the accessory chromosome had turned 90° and was situated parallel to the spindle. In one plant, the orientation of the spindle in the pollen grain often deviated more or less from the normal, which should be such that the generative nucleus will attain a position near the wall opposite to the pore. When the deviation from this normal orientation was large, the process of differentiation into a generative and a vegetative nucleus was upset, and the lagging accessory chromosome appeared to have difficulties in arriving at any one of the poles. This is in line with observations made by, among others, LA COUR (1949).

3. Inheritance of the accessory chromosomes

In Table 15, the results of crosses involving plants with different numbers of accessory chromosomes are given.

The data confirm the cytological observations on the non-disjunction in the pollen grains, rejecting the possibility of a similar mechanism on the female side. In the crosses 1—2/55 and 7—8/55, both of the type 1×0 and reciprocal, the loss of accessory chromosomes is pronounced in both directions. Plant 22 was not studied cytologically, but in plant 49 about 17 per cent of the tetrads had a micronucleus in one of the cells. As reflected by the cross, the elimination is thus much larger than was indicated by the cytological study. The two crosses

TABLE 15. *Inheritance of accessory chromosomes in Alopecurus pratensis.*

Parents				F_1										Total number of plants	M. acc. chr. per plant
Crosses	Plant Nos.		Number of acc. chr.	Number of accessory chromosomes											
	♀	♂	♀ ♂	0	1	2	3	4	5	6	7	8	9		
1/55	22×29		1×0	26	3	—	—	—	—	—	—	—	—	29	0.10
2/55	29×22		0×1	34	13	5	—	—	—	—	—	—	—	52	0.44
7/55	49×60		1×0	33	14	—	—	—	—	—	—	—	—	47	0.30
8/55	60×49		0×1	28	—	9	—	—	—	—	—	—	—	37	0.49
5/55	26×28		2×0	5	35	—	—	—	—	—	—	—	—	40	0.88
6/55	28×26		0×2	10	9	28	1	—	—	—	—	—	—	48	1.42
9/55	73×75		4×4	—	—	2	2	3	6	13	2	1	—	29	5.24
10/55	75×73		4×4	—	—	3	—	3	5	33	—	2	1	47	5.66
11/55	35×36		6×0	—	—	13	27	7	—	—	—	—	—	47	2.87
12/55	36×35		0×6	—	—	1	2	27	3	27	1	2	—	63	5.02

now mentioned also illustrate the variation in effectiveness of the process of non-disjunction. In the cross 5—6/55 the meiotic elimination of accessory chromosomes is less marked due to bivalent pairing. The consequences of the mechanism of non-disjunction are best illustrated by the last cross. In spite of the relatively high number of accessory chromosomes in plant 35, the meiotic elimination is very slight.

4. Discussion

The result of the collections suggests that the frequency of accessory chromosomes in south Swedish populations of *Alopecurus pratensis* is at least as high as in *Festuca pratensis* (BOSEMARK, 1956 a). Further, there is reason to believe that this also is the case in some parts of Finland and Germany (RANCKEN, 1934; WÖHRMAN, 1955).

The meiotic behaviour of the accessory chromosomes in *A. pratensis* largely corresponds to that in the majority of the grasses so far studied. The capacity of the accessory chromosomes to form a nucleolus of its own is, however, a new property. As it must be considered proved that the nucleolar material is formed along the entire chromosome complement and is only collected at the site of the nucleolar organizer (VINCENT, 1955), the addition of such an organizer might be of little importance to the plant as a whole. It is possible that it is useful to the accessory chromosome, helping it to reproduce if isolated from the

other chromosomes in a micronucleus (La Cour, 1952; Mc Leish, 1955). Fernandes and Serra (1944) have shown that in *Narcissus* the size of the ordinary nucleolus increases with an increasing number of heterochromatic accessory chromosomes, but it is doubtful if this is the case in *Ranunculus ficaria* (Mc Leish, 1955).

The results of the crosses indicate that the mechanism of non-disjunction is, at least in some genotypes, a very effective instrument for maintenance and spreading of the accessory chromosomes in *A. pratensis.*

F. Phleum nodosum

Phleum nodosum L. is a diploid Timothy-grass, relatively common in most parts of Europe (Nordenskiöld, 1945; Hubbard, 1954). The chromosome number $2n=14$ has been counted by, among others, Gregor and Sansome (1930), Müntzing (1935), and Nordenskiöld (1937, 1941, and 1945). Nordenskiöld (1945) has also briefly described meiosis in *Phleum nodosum.* None of the above mentioned anthors have reported individuals with »fragments» or accessory chromosomes in this species.

1. Material collected

The original plants were collected in five populations in the Province of Skåne and on the island of Öland. All plants had 14 normal chromosomes, but five plants from two populations were found to have in addition 1—4 accessory chromosomes (Table 16). These two populations were located close to each other in the vicinity of Alböke on the island of Öland, and grew on the poor rocky pasture land (in Swedish called *alvar*) typical of the island of Öland (Sterner, 1934; Bosemark, 1956 c).

TABLE 16. *Frequency of accessory chromosomes in spontaneous material of Phleum nodosum from Sweden.*

Sample Nos.	Number of plants with respective numbers of acc. chr.					Total number of plants	% plants with acc. chr.
	0	1	2	3	4		
12/54	7	—	—	—	—	7	
62/54	7	1	1	—	1	10	
63/54	6	—	2	—	—	8	
75/54	3	—	—	—	—	3	
79/54	12	—	—	—	—	12	
Total	35	1	3	—	1	40	12.5

2. Cytological observations

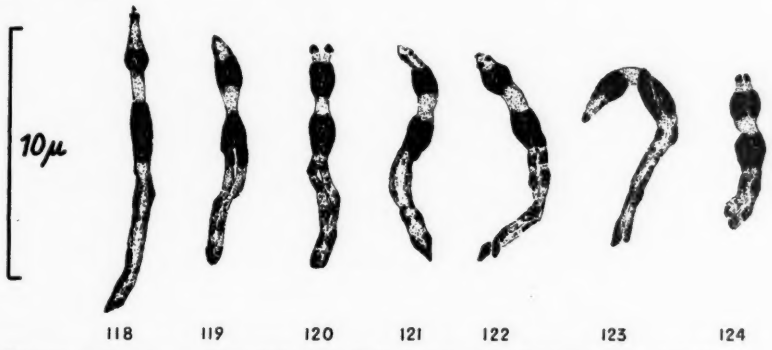
The size and shape of the accessory chromosomes in root-tip metaphase plates (Figs. 130, 131) correspond completely with that of the standard accessory chromosome in *Phleum phleoides* (BOSEMARK, 1956 c). The position of the centromere is often impossible to locate in root-tip metaphase plates, but at pachytene of meiosis and at the first pollen mitosis it can be seen to be sub-terminal. Also the normal complement looks very similar in the two species with the exception that the satellite of the nucleolar chromosome in *P. nodosum* is about three times as large as that in *P. phleoides*. The number of accessory chromosomes seems to be constant for an individual and the same numbers are present in both root-tips and anthers. With respect to the heterochromaty of the accessory chromosomes in resting nuclei and at early prophase in root-tips, it has not been possible to arrive at a definite conclusion as there are large heterochromatic knobs on probably all the ordinary chromosomes. However, with an increasing number of accessory chromosomes, the amount of positively heteropycnotic material appears to increase correspondingly.

Meiosis was studied in seven plants. One of them had $2n=14+1$ acc., two had $2n=14+2$ acc., and one had $2n=14+4$ acc. The remaining three plants had no accessory chromosomes. To begin with, the behaviour of the ordinary chromosomes will be briefly described. At pachytene pairing appears to be complete although individual chromosomes could not be followed in detail. Most of the chromosome arms were seen to have intercalary or terminal knob formations some of which are very marked (Figs. 125, 126). At diakinesis, the pairing behaviour varied very much between plants as illustrated by Table 17. The high frequency of univalents in plant 5 probably depends on non-homology due to a reciprocal translocation as indicated by the rare occurrence of trivalents and tetravalents, although a genetically determined reduction in chiasma frequency may also contribute to this situation.

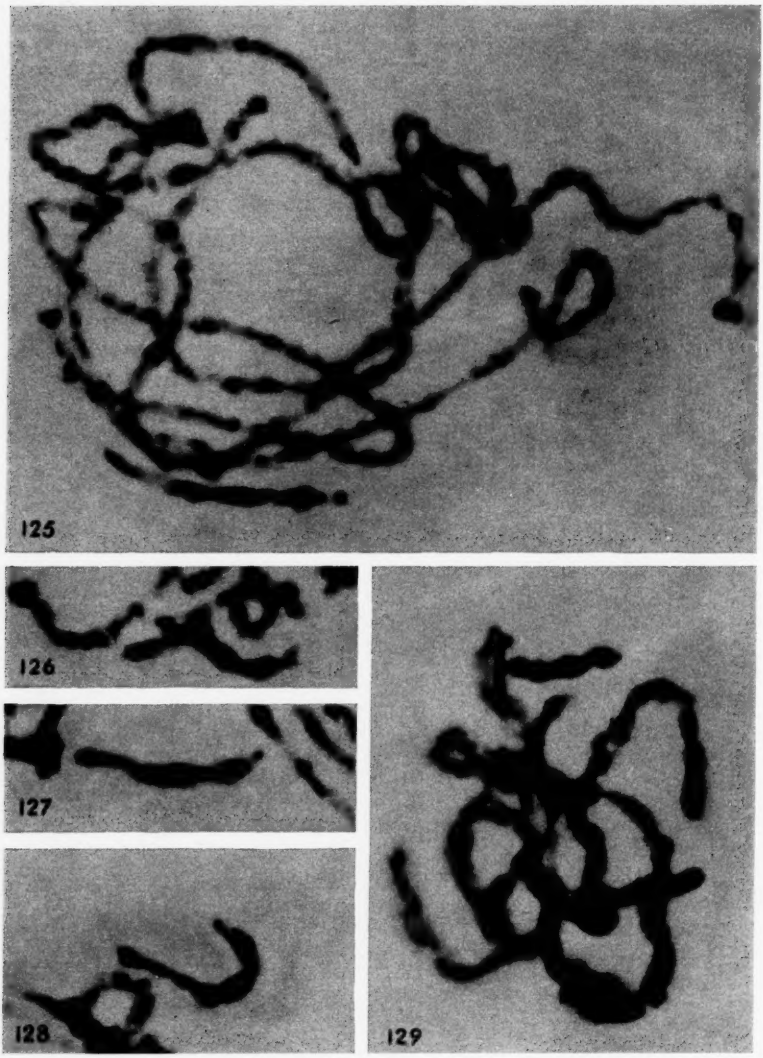
TABLE 17. *Pairing behaviour of the ordinary chromosome complement at diakinesis in three plants of Phleum nodosum.*

Plant Nos.	Chr. number	Configurations						Total number of cells
		$1_{IV}+5_{II}$	$1_{III}+5_{II}+1_{I}$	7_{II}	$6_{II}+2_{I}$	$5_{II}+4_{I}$	$4_{II}+6_{I}$	
5	14+1 acc.	2	1	52	30	5	2	92
10	14+2 acc.	—	—	62	—	—	—	62
2	14+4 acc.	2	1	24	4	—	—	31

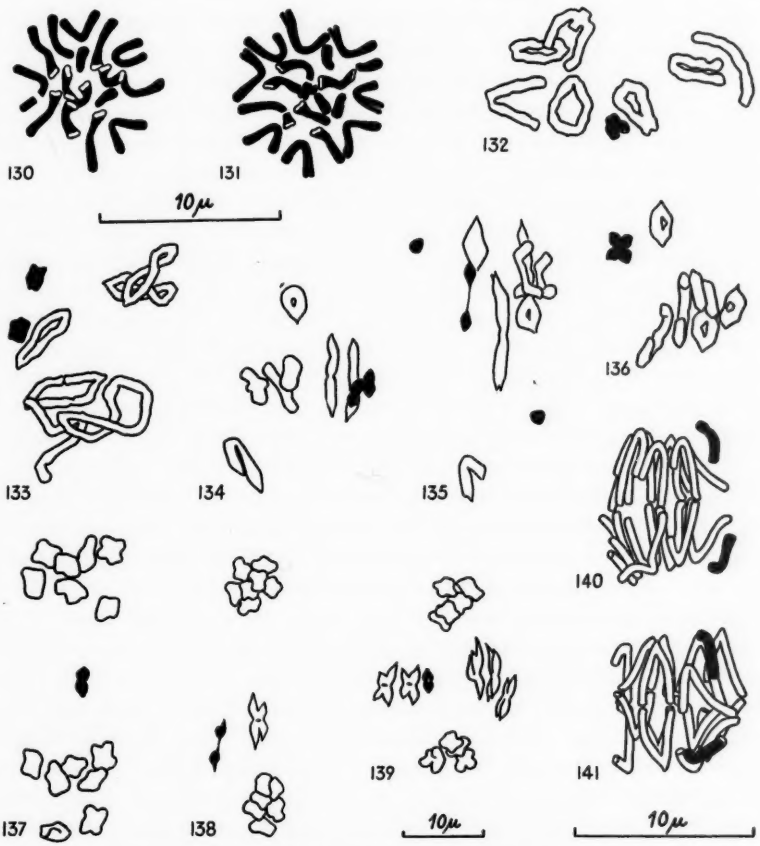
At metaphase I in plant 5, and to a certain degree also in plant 2, the number of rod bivalents was markedly higher than in for instance plant 10. It was observed that these rod bivalents were very often stretched out in their full length, sometimes with clearly pointed ends, although the centromere in all the ordinary chromosomes has a median or sub-median position (Figs. 134, 135). A close examination of metaphase I in plants without accessory chromosomes and with nearly exclusively ring bivalents revealed that even in these the ends often showed neo-centric activity, part of them being more or less drawn out and directed towards the poles. In plants with univalents from the ordinary complement, these often divided at late anaphase I; and in such cases, the arms of the daughter univalents were nearly always directed towards the poles (Figs. 138, 139). The figures are identical with those of dividing univalent accessory chromosomes as described in this paper and previously observed also in *Festuca pratensis* (BOSEMARK, 1956 b). At metaphase II, the chromosome arms are very often drawn out and directed towards the poles. At anaphase II, they move towards these with one or both ends leading. The manifestation of the neo-centric activity varies between plants, but was observed in all individuals studied irrespective of the presence of accessory chromosomes. As a matter of fact, this phenomenon was most pronounced in a plant with very regular metaphase pairing and lacking in accessory chromosomes. This activity of the chromosome ends, which is very likely a common property of the chromosomes of *P. nodosum*, strikingly resembles the behaviour of the chromosomes in *Elymus Wiegandii* (VILKOMERSON, 1950).



Phleum nodosum. — Figs. 118—123. Paired accessory chromosomes at pachytene. — Fig. 124. Paired accessory chromosomes at diplotene.



Phleum nodosum. — Figs. 125—128. Paired accessory chromosomes at pachytene. — Fig. 129. Paired accessory chromosomes at diplotene. — ×3210.



Phleum nodosum. — Figs. 130, 131. Root-tip metaphase plates. — Fig. 130. $2n=14+2$ acc. — Fig. 131. $2n=14+4$ acc. — Figs. 132—139. Meiosis in plants with various numbers of acc. chr. — Fig. 132. Diakinesis in a plant with $2n=14+2$ acc. — Fig. 133. Diplotene — diakinesis in a plant with $2n=14+4$ acc. The plant is probably heterozygous for a reciprocal translocation. — Figs. 134—136. Metaphase I in a plant with $2n=14+4$ acc. Note the terminal neo-centric activity of the normal chromosomes seen especially well in Fig. 135. — Figs. 137—139. Anaphase I in plants with $2n=14+1$ acc. In Figs. 138 and 139 the terminal neo-centric activity in the normal chromosomes is clearly seen in the dividing univalents. Also the accessory chromosome very likely behaves in the same manner (Fig. 138). — Figs. 140, 141. Anaphase of the first pollen mitosis showing normal division of the acc. chr.

Returning to the accessory chromosomes, their morphology was studied at pachytene (Figs. 118—123, 125—128). It was considered to be of special interest to compare the accessory chromosomes in *P. nodosum* with those already studied in *P. phleoides*. As was pointed out previously, the position of the centromere coincides in the two species and is sub-terminal. In *P. phleoides*, the short arm consists of two adjacent, generally easily distinguishable heterochromatic segments. Sometimes a small, pointed, weakly staining tail is seen at the distal end. In *P. nodosum* the short arm always seems to consist of only one relatively large heterochromatic block with a marked distal end, showing one or two rather large pairs of chromomeres.

The long arm of the accessory chromosome in *P. phleoides* can be divided into two halves of about equal length. The proximal half consists of three generally very clearly observable heterochromatic segments. The one next to the centromere is the smallest. The distal half of this arm is largely euchromatic, but contains smaller heterochromatic blocks or clusters of chromomeres. The border between the two halves of the arm is marked by an attenuated, weakly staining region. Also in *P. nodosum*, the long arm can be roughly divided into a hetero- and a euchromatic part. However, in this case, the proportion between the two halves is not 1: 1 but rather 1: 2, the heterochromatic part adjacent to the centromere thus being only half as long as the euchromatic. The heterochromatic region seems largely to be composed of one single block. The rest of the arm shows the same structure as the distal half of the long arm in *P. phleoides*. The difference between the accessory chromosomes in the two species is obvious also at diplotene.

In the plants with two accessory chromosomes, these generally formed a bivalent at diakinesis, whereas in the plant with four such chromosomes, all were associated in about 50 per cent of the cells observed. The corresponding figure for two bivalents was 45 per cent. In the remaining cells, one bivalent and two univalents were observed. Pairing between accessory chromosomes and members of the ordinary complement was not seen.

At metaphase I in the plant with one accessory chromosome, this had moved to the equatorial region in 82 cells out of 95. In plant 10, having two accessory chromosomes, these were situated on the equator in 100 out of 106 cells. In most of these 100 cells it could be observed that the accessory chromosomes formed a bivalent, but some cells were not quite clear and might have had two univalents hidden by the

ordinary bivalents. In the plant with four accessory chromosomes, no counts were made due to frequent clumping of the normal bivalents, but univalents were rather frequent (Fig. 135).

At anaphase I, paired accessory chromosomes disjoin normally, while univalents generally lag between the poles and divide later on. In the plant with one accessory chromosome, this was left between the poles in 48 of 61 cells, but at telophase I lagging accessories were seen at various distances from the main nuclei in only 12 cells of 69. At the second division, rather many laggards were observed in this plant as well as in the one with four accessories. At telophase II, most of these had reached the poles, and tetrad micronuclei were observed only in a few cases. However, in *P. nodosum* as in *P. phleoides*, the frequency of tetrad micronuclei observed is probably not a reliable measure of meiotic elimination. In both species, the boundaries of the main nuclei are indistinct and the micronuclei weakly stained. Many micronuclei might also have been resorbed in the cytoplasm before the tetrad stage.

The first pollen mitosis could be studied in only two plants with accessory chromosomes. In none of these plants were lagging accessory chromosomes seen between the two anaphase groups at the first division. A close study of a number of early anaphases, where all the chromosomes could be identified, verified that the accessory chromosomes undergo a normal division (Figs. 140, 141). This was a rather unexpected observation since in *P. phleoides* the mechanism of directed non-disjunction appears to be very efficient in the plants studied by the author (BOSEMARK, 1956 c). Although only two plants were cytologically examined in *P. nodosum*, the results of the crosses (see below), which involved other plants, confirm the cytological observation. However, all the plants with accessory chromosomes originated from two adjacent populations, and it cannot be excluded that they represent one extreme in a series ranging from absence of the capacity for non-disjunction to an efficiency comparable with that in *P. phleoides*.

3. Inheritance of the accessory chromosomes

Three reciprocal crosses were made in the usual way. The results of the chromosome counts are given in Table 18. In none of the crosses is there the slightest indication of a post-meiotic non-disjunction mechanism. In two of the crosses, *viz.* 1—2/55 and 3—4/55, the loss of accessory chromosomes appears to be more pronounced on the male than on the female side. In the first cross, however, the difference between the reciprocals with respect to the average number of accessory chromoso-

TABLE 18. *Inheritance of accessory chromosomes in Phleum nodosum.*

Parents				F_1				Total number of plants	M. acc. chr. per plant
Crosses	Plant Nos. ♀ ♂	Number of acc. chr. ♀ ♂		Number of acc. chr. 0	1	2	3		
1/55	5×14	1×0		23	38	1	—	62	0.65
2/55	14× 5	0×1		23	14	—	—	37	0.38
5/55	10×17	2×0		3	21	1	—	25	0.92
6/55	17×10	0×2		2	11	—	—	13	0.85
3/55	2×19	4×0		1	21	33	1	56	1.61
4/55	19× 2	0×4		2	24	6	—	32	1.13

mes per progeny plant is also partly due to an excess of plants with one accessory chromosome. It cannot be excluded that this deviation from the expected 1:1 distribution depends on some kind of gametic selection. Further crosses will have to be made to settle this matter. In the cross involving the plant with four accessory chromosomes (3—4/55), the apparent loss of accessory chromosomes might be partly due to self-fertilization although this was not indicated by the very slight seed-setting after isolation of two panicles.

4. Discussion

A comparison between the accessory chromosomes now described for *P. nodosum* and those in *P. phleoides* (BOSEMARK, 1956 c) has revealed both similarities and dissimilarities between these chromosomes in the two species. Although the pachytene analyses point to rather pronounced differences in the morphology of the accessory chromosomes at this stage, the over all organization and the similarity in size and appearance in somatic cells still suggests a common origin.

The most striking dissimilarity is the lack of non-disjunction at the first pollen mitosis in *P. nodosum*. Although this difference, as well as others, should for the present be strictly limited to the small material studied by the author, they invite further speculation. In *P. phleoides* (BOSEMARK, 1956 c), it was shown that loss of the short arm, as well as the distal largely euchromatic part of the long arm of the standard type of accessory chromosome does not markedly affect the functioning of the non-disjunction mechanism. This indicates that the seat of this mechanism is in the highly heterochromatic part of the long arm. As the main difference between the accessory chromosomes

in *P. phleoides* and *P. nodosum* appears to be in this very segment, it is possible that this difference is responsible for their different behaviour at the first pollen mitosis.

If the morphology of the accessory chromosome described here for *P. nodosum*, as well as their lack of non-disjunction, is found to be of general occurrence and a common origin is postulated, the question then arises as to which of the two types of accessory chromosomes is the primary one. *P. nodosum* might be a species where accessory chromosomes are on their way to disappear due to loss of the capacity for numerical increase and largely unfavourable effects. However, it is also possible that the accessory chromosome in *P. phleoides* represents a development not yet attained in *P. nodosum*. These questions can be tackled in different ways and would, even if only partly solved, give very valuable information.

The peculiar behaviour of the normal chromosomes in *P. nodosum* also deserves a more careful study. It would be of great interest to know with certainty if this is a general characteristic of the chromosomes in this species, and if so, how much it is influenced by the genotype and by external environmental factors. Also the possible connection between the knobs and the neo-centric activity (VILKOMERSON, 1950; RHOADES, 1952; RHOADES and VILKOMERSON, 1952; BOSEMARK, 1956 c) and the possible advantages of such a mechanism need to be clarified.

IV. GENERAL DISCUSSION AND CONCLUSIONS

Although the observations reported here are largely restricted to the cytological behaviour and the mode of transmission of the accessory chromosomes — and in certain respects have a limited reach — they have given additional information about the frequency of accessory chromosomes in the grasses. The mere fact that accessory chromosomes were found in six of eleven species sampled, despite limited sampling, indicates that they are very common.

The possibility of a geographical distribution of the accessory chromosomes within a species correlated with certain ecological factors, as clearly suggested by the studies in *Festuca pratensis* and *Phleum phleoides* (BOSEMARK, 1956 a and c), will make future careful population studies a useful tool in research which aims at a better understanding of the whole problem of accessory chromosomes. The species studied by the author, reported here and elsewhere, belong to three tribes, *viz. Festuceae*, *Aveneae* and *Agrostideae*. However, the differences in beha-

viour of the accessory chromosomes is equally great within as between tribes. It is very likely that within a genus the uniformity in behaviour is on a whole greater, but as yet our knowledge is too limited to allow a definite conclusion even in this respect.

In spite of the obvious differences between the accessory chromosomes in different species of *Gramineae*, these chromosomes must, at least at present, be considered to form a fairly homogeneous group. This is illustrated by Table 19.

The main questions in connection with research on accessory chromosomes in plants as well as in animals have always concerned the origin and selective value of these chromosomes. Fully satisfactory answers to these questions have never been given even for a single species. The present study has added very little to the solution of these questions. However, it has provided material to show that the majority of accessory chromosomes in grasses are heterochromatic and that they have a rather specialized, although extensively uniform, organization. To achieve their possible purposes, or even to be tolerated, they must obviously fulfill certain requirements which might be correlated with a special organization and morphology. Every change in an accessory chromosome is tested not only on the basis of the consequences of this change to the cell in which it is present but also on the basis of what it means to the accessory chromosome itself. Thus certain changes might be rejected because they have too unfavourable an effect on the cell, even if contributing to a better maintenance and spreading of the accessory chromosome, and vice versa.

It has also previously been pointed out by DARLINGTON and UPCOTT (1941), ÖSTERGREN (1947), and DARLINGTON (1956) that accessory chromosomes must have a special cycle of evolution, different from that of ordinary chromosomes. The evolution of an efficient accessory chromosome probably involves a rather delicate balancing of different needs. The fact that in the grasses there seems to be only one, quite dominant, basic type of accessory chromosome for each species, and often having a wide geographical distribution, suggests that the limits set by these requirements are rather narrow. The occurrence of the same type of accessory chromosome in populations from widely separated areas within the distribution range of a species, as for instance in rye (MÜNTZING, 1950, 1954), might also involve a common, perhaps ancient, origin of the particular accessory chromosome.

As the accessory chromosomes can be entirely dispensed with, but still exist in a certain, although varying portion of the individuals in a

TABLE 19. *Characteristics of accessory chromosomes in grasses.*

Tribe	Species	Smaller than the ordinary chromosomes	Heterochromatic	Constancy in number		Largely eliminated in vegetative tissue	Pairing at meiosis		Univalents divide at A I	Non-disjunction	
				Root-tips	Anthers		With ordinary chromosomes	With each other		Male side	Female side
		1	2	3	4	5	6	7	8	9	10
Maydeae	*Zea mays*	+	+	+	+	−	−	+	+	+[3]	−
Andropogoneae	*Sorghum purpureo-sericeum*	(+)[1]	+	see 5	+	+	−	+	−	+[4]	−
Festucae	*Festuca pratensis*	+	+	+	+	−	−	+	+	+	−
"	" *arundinacea*	+	+	+	+	−	−	+	+	+	−
"	*Poa alpina*	+	+	see 5	−	+	−	+	−	−	−
"	" *trivialis*	+	+	(+)[2]	−	−	−	+	+	−	−
"	*Briza media*	+	+	+	+	−	−	+	+	+	−
Aveneae	*Holcus lanatus*	+	+	+	+	−	−	+	−	+	−
"	*Anthoxanthum aristatum*	+	+	+	+	−	−	+	−	+	−
Hordeae	*Secale cereale*	+	(+)[2]	+	+	−	−	+	+	+	+
Agrostideae	*Phleum phleoides*	+	+	+	+	−	−	+	+	+	−
"	" *nodosum*	+	+	+	+	−	−	+	+	(−)[2]	−
"	*Alopecurus pratensis*	+	+	+	+	−	−	+	+	+	−

[1] Various size classes.
[2] Not marked or doubtful.
[3] Second pollen mitosis followed by directed fertilization.
[4] In supernumerary divisions in the vegetative nucleus.

population, it is of greatest interest to find out what are the mechanisms which bring about this apparently balanced condition.

Being always subjected to a varying degree of meiotic, sometimes also mitotic elimination, the accessory chromosomes would inevitably be lost in succeeding generations if the elimination were not compensated for by some other factors. The first thing to suggest is of course that, at least under certain conditions, natural selection favours plants with a moderate number of accessory chromosomes, thus keeping the balance (DARLINGTON and THOMAS, 1941; DARLINGTON and UPCOTT, 1941; DARLINGTON, 1956). Attempts have been made to test this hypotesis for the accessory chromosomes in grasses. The studies by RANDOLPH (1941) in maize, MÜNTZING (1943, 1954) in rye and ÖSTERGREN (1947) in *Anthoxanthum* have, however, all failed in finding any positive effects of the accessory chromosomes in these species, irrespective of the number present. On the other hand, it has been demonstrated beyond doubt, that in these species, as in *Festuca* (BOSEMARK, 1957), high numbers of accessory chromosomes, at least, have deleterious effects on fertility as well as vegetative development.

With the detection of the non-disjunction of the accessory chromosomes in rye (MÜNTZING, 1946; HÅKANSSON, 1948 a) and *Anthoxanthum* (ÖSTERGREN, 1947), a powerful mechanism for maintenance and spreading of these accessory chromosomes was revealed, seemingly well capable of counterbalancing the meiotic loss. As essentially the same mechanism is present in *Sorghum* (DARLINGTON and THOMAS, 1941) and maize (ROMAN, 1947, 1948) and has been found in six more species by the present author, it is obviously widespread among the grasses. It is possible that also other mechanisms giving the same ultimate result do exist within the family; *e. g.* in *Poa trivialis*. MÜNTZING (1943, 1945, 1946 a, 1954), on the basis of his studies of the genetical effects and inheritance of accessory chromosomes in Swedish rye, largely rejects the possibility of a positive selective value of the accessory chromosomes in this species and considers them to be maintained exclusively as a consequence of their mechanism of numerical increase. ÖSTERGREN (1945, 1947), on the same grounds put forward the hypothesis of the parasitic nature of accessory chromosomes.

When first published, the necessary condition for the application of this hypothesis, *viz.* a mechanism for numerical increase, was only definitely established for the accessory chromosomes in rye, *Anthoxanthum*, and *Sorghum*. For this reason and because the evidence for the correctness of such a hypothesis must always ultimately rest on

mainly negative experimental results, the hypothesis of the parasitic nature of accessory chromosomes has not generally been accepted as sufficient to explain the widespread occurrence of accessory chromosomes. Thus, DARLINGTON (1956), among others, argues entirely in favour of the accessory chromosomes being useful, although not indiscriminately so. Although DARLINGTON seems to underestimate the significance of the non-disjunction mechanisms for the maintenance of accessory chromosomes in grasses, the present author is inclined to accept at least some of his basic ideas. This point of view is based on: (1) available indications of positive effects of accessory chromosomes in some species and, (2) the information obtained on this question by population studies.

(1) Experiments carried out by the author in *Festuca pratensis* (BOSEMARK, 1957), suggest that the effect of low numbers of accessory chromosomes on the vegetative development is not uniform, but probably largely dependent on the genotypic constitution of the material. Thus, in some materials, one and two accessory chromosomes seem to have a slightly stimulating effect on plant growth, while in other materials, they have no measurable effect. The data, however, are still insufficient for definite conclusions, and further experiments — under controlled environmental conditions — will have to be done. However, in *Centaurea Scabiosa* (*Compositae*) FRÖST (1954) has found that in some genotypes, and under the conditions of cultivation applied by him, plants with low numbers of accessory chromosomes have a significantly higher average plant weight than closely related plants without such chromosomes. Thus, if growth rate and vegetative production under artificial conditions — which do not involve competition — may be taken as a measure of fitness, these data suggest that, under certain conditions, there are some species in which accessory chromosomes are advantageous.

In *Sorghum* (DARLINGTON and THOMAS, 1941), the accessory chromosomes stimulate the vegetative nucleus in the pollen grains to polymitotic divisions. This is, however, often followed by a disastrous drop in pollen fertility. As the accessory chromosomes in this species are eliminated from root-tips and probably other vegetative organs and are maintained only in the germ-track, they seem to agree with the idea of a parasitic existence. DARLINGTON (1956), however, believes that they have a specialized value for the reproductive processes. This idea is supported by the studies in *Poa alpina* (MÜNTZING, 1948 e, 1954) in which species the apparent lack of plants without accessory chromoso-

mes, in some biotypes, has been taken as an indication that they have some important functions.

Knobs and B-chromosomes in maize have some important characteristics in common. The inverse relationship between number of knobs and B-chromosomes in maize from American indians (LONGLEY, 1938), led DARLINGTON and UPCOTT (1941) to the conclusion that the B-chromosomes have taken over the rôle of the knobs in nucleic acid metabolism, thereby providing a more flexible means of regulating these processes for the nucleus as a whole. Leaving aside the rôle of the knobs and heterochromatin in nucleic acid metabolism, it is obvious that the reasoning can be applied equally well to other functions. Thus, all processes which ultimately affect the selective value of the individual, and are also partly or wholly governed by polygenes, may be considered to belong here. Recently, DARLINGTON (1956) has also pointed out that the value of accessory chromosomes might be to boost variability and adaptability of the species. The advantages and disadvantages of a system which involves a compromise between the needs for immediate fitness and adaptability are obvious. The effect of the accessory chromosome, *per se*, will determine how rapidly their number must be capable of changing in a population to make the system workable.

(2) The results of the population studies have shown not only that accessory chromosomes occur in many grasses, but also, that they are very common in some species. This fact alone may be considered an indication of their usefulness rather than the contrary. The geographical distribution of plants with accessory chromosomes within some species (BOSEMARK, 1956 a; FRÖST in MÜNTZING, 1954) makes it very likely that the effect of the accessory chromosomes is influenced by the genotypical constitution of the population as well as external environmental factors. The variation in frequency of accessory chromosomes between different geographical areas, as revealed in the above mentioned studies, probably covers only a part of the total variation. The extremely low frequencies of accessory chromosomes reported in cultivated materials of rye from Europe, supplemented by recent reports of fairly high frequencies in more primitive strains from Asia Minor and very high frequencies in some varieties from eastern Asia (MÜNTZING, 1954 and personal communication), point to this. The lack of evidence of any positive effects of the accessory chromosomes in MÜNTZING's Swedish rye material should be considered in the light of these results. Before careful studies of the genetic effect of accessory

chromosomes in the primitive Asiatic rye varieties have been undertaken — preferably in their native environment — the results for highbred European varieties should not be generalized.

Still much research remains to be done on accessory chromosomes in grasses, as well as in other families, before we have sufficient knowledge to understand their function, their selective value, and their origin.

SUMMARY

(1) The cytology and transmission of accessory chromosomes in *Festuca arundinacea*, *Poa trivialis*, *Briza media*, *Holcus lanatus*, *Alopecurus pratensis*, and *Phleum nodosum* was studied.

(2) Of all species except *F. arundinacea*, the material consisted of plants collected in natural populations in southern Sweden. The material of *F. arundinacea* originated from seeds collected from a natural population in England.

(3) The sampling has not been extensive enough to permit the drawing of definite conclusions regarding the frequency of plants with accessory chromosomes in the different species. The data indicate, however, that in some of the species accessory chromosomes are common within the sampled areas.

(4) In all the species the accessory chromosomes are smaller than the ordinary ones and easily distinguishable. They are very likely all heterochromatic.

(5) In all but one species the number of accessory chromosomes appears to be constant in different parts of the individual. In *Poa trivialis* there is a variation in number of accessory chromosomes between as well as within flowers from one and the same plant. Occasionally there was even a variation in the number of accessory chromosomes in the root system.

(6) The morphology of paired accessory chromosomes during pachytene was studied in all species. The position of the centromere and the distribution of the heterochromatic segments could be determined. In *P. trivialis*, a peculiar end to end association of paired accessory chromosomes was observed at this stage.

(7) The pairing of the accessories at metaphase I varies between as well as within species, but bivalents were observed in all of them. No pairing between accessory chromosomes and members of the ordinary complement was observed in any of the species.

(8) In all species except *Holcus lanatus*, univalent accessory chro-

mosomes left between the poles at early anaphase I are capable of dividing at late anaphase I — telophase I. Even in *H. lanatus*, the univalents show marked tendencies to division. However, complete separation of the chromatids was never observed.

(9) In all the species except *Poa trivialis*, dividing univalent accessory chromosomes display a more or less marked terminal neo-centric activity, the ends of the chromatids generally being drawn out and directed towards the poles.

(10) The meiotic elimination is variable, but on the whole moderate.

(11) In four of the six species, the accessory chromosomes have the ability to undergo non-disjunction directed to the generative nucleus at the first pollen mitosis. In *Phleum nodosum* and *Poa trivialis*, no similar mechanisms which will result in an increase in the number of accessory chromosomes have as yet been ascertained. However, the material studied in both species is very limited; furthermore, in *P. trivialis*, it cannot be excluded that the variation in the panicles involves a mechanism of numerical increase.

(12) Crosses between plants with different numbers of accessory chromosomes were made in all six species. The cytological observations on non-disjunction of the accessory chromosomes in the pollen grains in *F. arundinacea*, *B. media*, *H. lanatus*, and *A. pratensis* were confirmed. The results of the crosses showed that in all the species the mechanism of numerical increase is restricted to the male side. On the whole, the cytological observations and the results of the crosses are in good agreement. The number of crosses analysed is insufficient for a general judgment of the effectiveness of the non-disjunction mechanism and its consequences on the maintenance of the accessory chromosomes in natural populations.

(13) A general discussion on accessory chromosomes in grasses is given. It is concluded that our knowledge is still too restricted to permit definite conclusions to be drawn regarding the origin, the genetical effects and the selective value of these chromosomes. Nevertheless, data have been obtained for some species which suggest that, in the case of certain genotypes under some environmental conditions, accessory chromosomes may be useful.

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MUTATIONS AND THE INTENTIONAL RECONSTRUCTION OF CROP PLANTS

By *DITER von WETTSTEIN*
GENETICS DEPARTMENT, FOREST RESEARCH INSTITUTE OF SWEDEN, STOCKHOLM
(Lecture delivered at the 5th Internat. Conference on Radiobiology, Stockholm, August, 1956).

FOR THE use of induced mutations in plant-breeding, knowledge of the mutation process and an understanding of the effects of different mutation types on the crop plant are necessary. Until recently the mutation process was considered to be completely at random. In barley, however, a control and direction of the mutation process can be demonstrated. It is possible to change the mutation spectrum of lethal and viable mutations considerably by using radiations with different ion density. Even mutations of high agronomical value as the erectoides types may be preferentially induced with radiations of high ion density. As to the lethal chlorophyll mutations a change of the spectrum can be achieved by varying the physiological stage in the seeds.

In comparing the effect of different chemical mutagens in barley two different types of changes in the mutation process are looked for: 1) The change of the mutation spectrum in the second generation using the lethal chlorophyll mutations as a test. 2) The separation of chromosome rearrangements and gene-mutations. A measure of the induced chromosomal aberrations is provided by the sterility of the first generation after treatment, and for gene changes the chlorophyllmutation-test is used, since most of these mutations represent factor mutations.

The change in the mutation spectrum with the mustards as compared with that obtained after irridiation implies an increase of *viridis* at the cost of *albina* types. Outstanding in this respect is the mutagenic effect of the nucleoside nebularine. The mutations induced by this substance are almost exclusively of the *viridis* type, whereas the mutation rate of the other chlorophyll lethals does not seem to increase much above the spontaneous level. Other compounds such as ethylene oxide give proportions of mutations which agree with those obtained after irradiation.

Comparing the efficiency of different mutagens in inducing chromo-

somal aberrations and mutations, the purine-derivative 8-ethoxycaffeine causes considerable chromosome breakage and high sterility in the first generation, whereas visible mutations seem to be induced only rarely. Nebularine on the other hand does not give rise to chromosome breakage, chromosome rearrangements and sterility in the first generation but enhances the factor mutation rate. The mustards and ethylene oxide induce aberrations and gene changes in a manner similar to that of ionizing radiations.

Thus, it seems possible to induce special types of mutations at will and there is no reason why this should not be applicable to vital mutations of use in plant-breeding. The significance of these results may be grasped when we have learnt the effects of different mutation types on viability and morphology.

It is easy to isolate translocation lines in barley. A close study of such lines shows that chromosomal rearrangements are of little importance in changing the morphology of a plant. Certainly some translocation lines may affect the vigour of a plant in a negative sens, for instance, as regards straw-length and kernel weight. But as a rule the translocation lines cannot be distinguished from the mother variety. All lines of X-ray and neutron translocations tested together give on an average the same yield as the original mother variety. These lines include a type homozygous for a translocation chromosome with two satellites, one at each end, a chromosomal type not known to occur in nature. Obviously, at least the barley species is extremely tolerant to a reorganization of the caryotype. Some of the reorganized caryotypes exhibit a yielding capacity surpassing that of the mother variety. Such a positive effect of chromosomal rearrangements will be of importance when we want to improve the yield of certain varieties without changing the agronomical properties mainly connected with the morphology of the plant. As regards crop plants we have to inquire how the reorganized caryotypes may influence competitive ability and the ecological response, as well as the physiology of producing organic matter and different reserve substances.

The ecological differentation in barley proceeds by factor mutations with or without accompanying drastic changes in morphology. An ecological fitness to drought was induced in the »late tall» mutant of barley, and even to some extent in the »bright greens». The erectoides mutations, on the contrary, are rather adapted to humid conditions. They may also be called nitrogen ecotypes since, relatively seen, they are highest yielding with an abundant nitrogen manuring. They can

tolerate and utilize more nitrogen than the mother varieties. The bright greens, on the other hand, react adversely: they prefer nitrogen poor soil.

There are many examples in higher and lower plants of a morphological differentiation by factor mutations, which at the same time are of importance to agriculture and drug production. We will confine ourselves here to discussing the problem of straw-stiffness in barley and wheat, since we think that this problem can be solved by an extreme mutational repattering of the straw morphology. The investigations have advanced to a point where it is possible to see how such a planned reconstruction may be achieved.

At first a test for straw-stiffness on a morphological basis was worked out with the aid of the extremely straw-stiff erectoides mutations. From these studies it is clear that the length of the individual internodes in the straw is of great importance for resistance to lodging. Short basal and long upper internodes are favourable. The number of straw-internodes should be kept at four to five. Further, an increase in cross-section area of the straw will improve the value of a market variety. By taking up these and other morphological characteristics for a given variety its straw-stiffness can be determined in relation to a known standard line within one season, and this even in a year without lodging in the field.

That selection and continued breeding has brought about a one-sided morphological architecture in different crop plants is best demonstrated by a comparison of wheat and barley. Within the two species the degree of straw-stiffness can be estimated from quantitative differences in the morphological traits named above. At the same time it appears that straw-stiffness in wheat rests on a rather ideal internode-number and distribution of internode-lengths. The cross-section area, however, lies far below that of the barley varieties studied. In barley, on the other hand, the straw-stiffness seems to rest entirely on the high cross-section area of the stem, whereas the distribution of internode-lengths is inferior. Thus, regarding straw-stiffness, we have to remake barley so as to resemble wheat in some traits, and vice versa.

Induced mutations are of special use in this respect. The erectoides mutations, appearing in 15 to 20 different loci, bring about a pleiotropic change in the construction of the straw which is in the direction of the programme. It is to be noted that one single mutation step may provide several traits beneficial to straw-stiffness. Not only the erectoides mutations, however, are of help in fullfilling this program. Early mutations may show a reduced number of internodes, and the analysed

giant mutation simulates a tetraploid, but without the reduced vigour of tetraploid barley. The cell-size in the giant mutant is generally increased. This circumstance can be used to increase the cross-section area and the thickness of the culm-wall. The erectoides mutations give an instructive example of mutation towards an increase in cell-size restricted to certain tissues, namely vascular bundles, parenchym and sklerenchym tissue in the culm without affecting the leave tissues and pollen-size. Other mutants too provide an excellent raw material for breeding towards high straw-stiffness. For instance the influence of the lignification process and the root system on the resistance to lodging is studied by means of specific mutants. No doubt crossing and recombination of different mutations will bring together different traits responsible for an extremely stiff culm. This will take some time, however, since relations of dominance and epistasy are often complex in drastic pleiotropic mutants of barley and we do not know the principles involved sofar.

Erectoides 23 at locus *a* is perhaps the most perfect mutant as to straw construction but it suffers from a small loss of yield compared to the weak-strawed but high productive mother strain Bonus. We are trying to remove this drawback by inducing rearrangements of the karyotype according to the principles outlined above, *viz.* the fact that in general translocations do not influence the morphology but give rise now and then to positive variants as to yield.

Another problem to be faced in mutation breeding is that of correlation. The occurrence of a barley straw having a high resistance to lodging is correlated with a dense spike, probably harmful to yield. New induced mutations of erectoides 23 exemplify the fact that such correlations may be broken. One mutant shows a reversion as to straw construction in the direction of the original mother variety Bonus, but it has retained the dense spike. Also the other type of break in the correlation has recently been found. In this type a lax ear — a partial reversion to the original strain Bonus — is combined with a culm construction, typical for straw-stiff erectoides types.

To repattern a crop plant by means of mutation is to start with the help of drastic or profound gene and chromosome changes, but for the final refinement of the phenotype the small mutations are certainly necessary. Their occurrence is easily demonstrated in the group of early mutants. They have been studied systematically with regard to ear-density. In this character they occur spontaneously in both directions, but as yet they have not appeared more frequently after irradiation.

Last but not least another type of mutants seems important in mutation breeding. Some of the so-called block-mutations used in *Neurospora* and other lower organisms might be helpful also in crop plants in order to increase sugar, oil or fat content. That such block-mutations are in principle to be found in barley too is shown by the chlorophyll lethal *xantha* (v. WETTSTEIN, 1954).

To sum up, mutation breeding may result in agronomical high productive types as shown by some X-ray varieties of white mustard, peas, peanuts and others. Mutation-breeding will be of value in combination with the other methods of plant-breeding: selection, crossing and polyploidy. The greatest significance of induced mutations lies no doubt in the fact that they provide a tool in the intentional reconstruction of the species and the crop plant. Personally, I consider that not only are the genetic resources for such a planned remake great and already now to a certain extent sufficient, but also that they will gain in importance by a successively more detailed information concerning the control and direction of the mutation process. At the same time, however, this reconstruction is at present limited by our inferior knowledge of morphology, anatomy and physiology of development in crop plants, especially in relation to their agronomical properties. Here, too, belongs the problem of competition and co-operation of genotypes in crop plants. It is an inspiring task to elucidate such problems one by one and to work out suitable tests for the improvement of individual traits. In this work the effects of single large mutations will be especially useful. This is further accentuated by the fact that every trait closely studied can be changed by induced mutation. Even entirely unexpected traits may arise. Moreover we cannot overlook the gains biochemistry has obtained through analysis of block-mutations.

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GENETICS AND THE SUBMICROSCOPIC CYTOLOGY OF PLASTIDS

By *DITER von WETTSTEIN*
GENETICS DEPARTMENT, THE FOREST RESEARCH INSTITUTE, STOCKHOLM

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CYTOGENETICS has come to mean a parallel, genetical and chromosomal analysis. The preference of the cytology of chromosomes is due partly to the eminent role they play in mendelian heredity and partly to the accessibility of chromosomal structure by light microscopy. The structure and differentiation of the other components in the cell, as for instance mitochondria and plastids, are more difficult to study with the light microscope and our knowledge about them has until recently been obtained largely by indirect methods.

In the last few years the electron microscope with its high resolution power and the development of suitable techniques of preparations has made it possible to approach directly the submicroscopic cytology, especially that of the plastids. This will help to provide a cytological basis for the rather advanced genetical analyses regarding extranuclear inheritance. This, of course, has to start with the descriptive analyses of the life cycle of plastids in plants and of mitochondria and other plasmatic components in both plants and animals. On the other hand the submicroscopic cytologist can immediately make use of genetically well defined and investigated specimens. These include genom mutations — lethal or viable — affecting the development and function of chloroplasts as well as different plasmatically inherited chlorophyll deficiencies. It is the aim of the present paper to summarize the facts of the submicroscopic cytology of plastids so far obtained as regards their bearing on the genetics of plastids. The discussion will be based mainly on the following studies: LEYON und v. WETTSTEIN, 1954; v. WETTSTEIN, 1954; STUBBE und v. WETTSTEIN, 1955; v. WETTSTEIN, 1957. For the used terminology the reader is referred to the last named paper.

THE CHLOROPLAST STRUCTURE IN DIFFERENT PLANT SPECIES

In the discussion on the genetical autonomy of the chloroplast, especially in *Oenothera*, it has been assumed that the quality of the chloroplasts even in related species may be fundamentally different (RENNER, 1936 a). Quantitative structural differences as to size of the grana in the chloroplasts of different tissues, of plants in different physiological conditions and of different species as well as genera are well known from light microscopical studies. What are then the similarities and dissimilarities of the submicroscopic chloroplast structures in different genera of thallophytes and cormophytes so far investigated?

In Fig. 1 completely differentiated chloroplasts of several genera are gathered. The plastids are in no way organells with a stationary structure. They show a so called monotropic development (cf. v. WETTSTEIN, 1957) which starts with proplastids in higher plants or some sort of elementary chromatophores in algae. Until they degenerate in old cells their inner structure is continuously shifting. In later stages this is generally accomplished by a multiplication of layers and lamellae. The full-grown or completely differentiated chloroplasts in this discussion represent the earliest stage, when photosynthesis can be performed, a stage that can be traced owing to the presence of accumulated assimilates within the chloroplasts.

The basic structure hitherto found in algae (for literature see LEYON, 1956, v. WETTSTEIN, 1957) consists of layers composed mostly of 4 lamellae with a thickness of 60 ± 20 Å. The structure of the *Fucus* chromatophore may serve as an example (Fig. 1). Even the big and complicated chromatophores of certain algae, as *Spirogyra* (STEINMANN, 1952; LEYON, 1954) or *Ankistrodesmus* (STEFFEN and WALTER, 1955) contain the same structural elements. The mechanisms of the division and multiplication of these layers and the chromatophores as found in *Fucus* make the formation of the most complicated chromatophores understandable in principle. So far none of the investigated thallophytes has revealed the type of grana differentiation characteristic of higher plants.

In the chloroplasts of higher plants the layer is a basic element too. The layers are of variable thickness, contain different numbers of lamellae and are in some species like barley connected to form a network. (Fig. 1). The number of layers in a chloroplast just having reached the full-grown stage is fairly constant. For barley they have

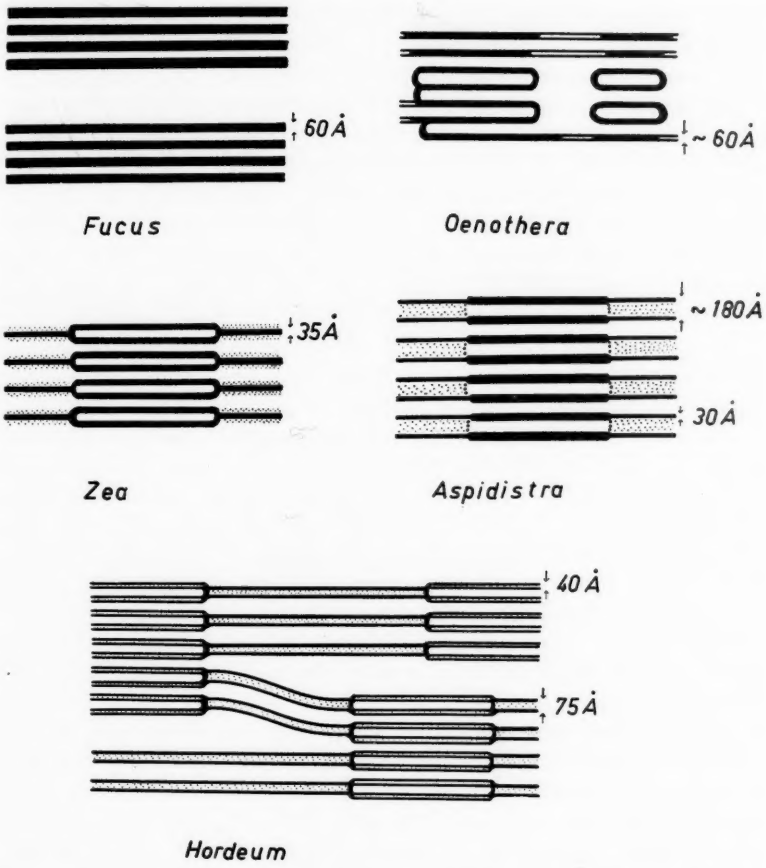

Fig. 1. Submicroscopic organization of chloroplasts in different plant genera (according to LEYON and v. WETTSTEIN, 1954; STUBBE and v. WETTSTEIN, 1955; HODGE, MC LEAN and MERCER, 1955, 1956; STEINMANN and SJÖSTRAND, 1955; LEYON, 1956; v. WETTSTEIN, 1957).

been counted at 6—12, for *Oenothera* at 7—10, for *Aspidistra* around 10 (LEYON, 1956), for tomato at 8—12 (LEFORT, unpublished) and for mosses at 5—10 (MENKE and MENKE, 1956). The number of layers increases when chloroplasts are getting older and bigger. Then the layer structure, so clearly visible in barley and maize, is no longer pronounced, not even in these objects (HODGE, MC LEAN and MERCER,

1956, v. WETTSTEIN unpublished). The arrangement of the grana structure has been discussed earlier by the author (v. WETTSTEIN, 1957). It was stated that in some objects as *Aspidistra* and *Chlorophytum* the grana columns can be of great height, eventually transversing the chloroplast as a whole in one single column, whereas in other objects as indicated for barley in Fig. 1 the grana columns are of restricted height and do not lie above one another. As has been pointed out for the maize chloroplast (HODGE, MC LEAN and MERCER, 1956) and confirmed in unpublished studies for barley, the older chloroplasts show a marked tendency towards pairing of their grana together with the disappearence of the layer structure. Thus this difference is not so much a principal one as a question of the physiological stage of the mature chloroplast and the time at which these stages are reached in different species.

The layers of the chloroplast are built up by lamellae and fine layers within these lamellae. The grana lamellae have a thickness of 40 to 60 Å (cf. Fig. 1). The individual grana lamella of barley could be resolved into two fine layers 10—20 Å in thickness. This has been independently shown to be true also for the maize chloroplast (HODGE, 1956; HODGE, MC LEAN and MERCER, 1956). As regards the *Aspidistra* chloroplast a composite structure of the grana lamellae is indicated (LEYON, 1956). The stroma lamellae in *Aspidistra* show a marked pairing. Their diameter is about 30 Å, whereas the stroma double lamellae have been measured to about 180 Å. In barley a stroma lamella is ca 75 Å thick and composed of two fine layers with ca 20 Å in diameter. Obviously the single stroma lamella of barley corresponds to to the double lamella of the stroma in *Aspidistra*. There is every reason for believing that the stroma lamellae of the maize chloroplast are composed like those of barley. Thus the basic difference between these two chloroplast types does not seem to lie in a different number and thickness of the lamellae and fine layers. The specific difference between the species is the degree of pairing of the 20—30 Å thick fine layers in the stroma or in other words, the mass density of the material between the fine layers varies. The grana areas have twice as many fine layers as the stroma areas, and seem structurally alike in the different species.

The structural organization of *Oenothera* chloroplasts looks at first sight quite different from those just mentioned. A similar structure has recently been found in young assimilating chloroplasts of tomato (LEFORT to be published). This organization can rather readily be

explained by the developmental picture of the chloroplast structure given below. It suffices here to say that tomato chloroplasts reach in older leaves a structure similar to that of *Aspidistra.*

In spite of the fact that certain definite differences between the submicroscopic structure of chloroplasts in higher plants have been obtained, the basic lamellar organization is the same in widely different genera. Regarding the ultimate molecular structure of the association between pigments, proteins and lipoids, it is found that the electron microscopically resolved fine layers with a thickness of 10—20 Å lend support to the idea of a monomolecular layer structure in contrast to the globular form of the postulated lipochromoproteid in the chloroplasts (cf. FREY-WYSSLING, 1955).

DEVELOPMENT AND CONTINUITY OF THE SUBMICROSCOPIC CHLOROPLAST STRUCTURES

It is generally agreed that in many algae the chromatophores multiply by fission and that the division of existing chromatophores is the prominent mechanism by which the chromatophores are handed over as organells from one generation to the next. For a certain strain of *Euglena* it was shown that the cells which have lost their chromatophores by prolonged cultivation in the dark, cannot regenerate them when the cells are placed in light again (LWOFF and DUSI, 1935). From such facts the general cytological theory of self-duplication of plastids has been developed. Note here, however, that the chloroplasts in *Nitella* develop from undifferentiated proplastids (HODGE, MC LEAN and MERCER, 1956), precisely as in the case of higher plants.

The investigations on *Fucus* have shown that the elementary chromatophores in egg cells and the vegetation point show a structure of layers and lamellae as described above. The multiplication of the layers and the division of the chromatophores were studied. The layers multiply by thickening and splitting. The chromatophores divide by the formation of pseudopode-like protrusions and successive constrictions form local parts of all layers. Thereby the layers are brought into close contact, combine and divide in a plane vertical to their longitudinal axes. This process starts in the innermost pair of layers and proceeds towards those of the periphery until all layers are separated. This results in the formation of new independent continuous layers and at the same time the chromatophore is divided into two daughter chromatophores. The elementary chromatophore which is capable of

multiplying, differentiates in the somatic parts of the thallus mainly by an increase in size and in number of layers.

The above referred processes are able to provide cytological evidence for the self-duplication of the chromatophore layers. I want to emphasize, however, that this does not mean a hereditary continuity that is independent of the nucleus. On the contrary I think there are facts reported which argue against such a genetic continuity of the chromatophore layers comparable to that of the chromosomes in the nucleus.

Certain strains of *Chlorella, Chlamydomonas* and *Euglena* can synthesize chlorophyll in the dark and in the light. Other strains can synthesize chlorophyll only in the light. This difference can be brought about by gene mutation (GRANICK, 1951). The ability to form chlorophyll in the dark is connected with the stability of the chromatophore layers. One such mutant in *Chlamydomonas* (SAGER and PALADE, 1954) which has lost its ability to form chlorophyll in the dark shows the absence of chromatophore layers in dark culture. Strains of *Euglena* and *Poteriochromonas* which cannot form chlorophyll without light loose their layers and lamellae in the dark rapidly. In their electron microscopical study WOLKEN and PALADE (1953) even state: the chromatophores »seem to vanish beyond recognition». In such cultures put to light again the chromatophores and their lamination are readily restored. It is of special interest, that the lamellate structure of the *Nitella* chloroplast (without grana differentiation) is developed during ontogenesis and that the continuous lamellae are formed by the fusion of short pieces of double lamellae (HODGE, MC LEAN and MERCER, 1956), a process, characteristic for the development of the submicroscopic organization of chloroplasts in higher plants.

These facts taken together disclose that the development of chromatophores and their submicroscopic structure in algae can start from small undifferentiated organells or perhaps from cytoplasmic structures. There are two trends appearing:

I. The development of the layered plastids starts with undifferentiated, possibly plasmatic, structures. This process is controlled by a great number of genes as shown in barley (v. WETTSTEIN, 1954; 1957). As long as these genes are capable of normal function, the enzymatic formation of pigments in the dark is dispensable, as well as the genes controlling the latter process. This type of plastid development is found generally in higher plants and occasionally in algae. Certain algae have at least the ability to develop their submicroscopic plastid structure

from an undifferentiated stage, even if the plastids normally divide by fission.

II. The mechanisms of the division of layered chromatophores and the multiplication of layers and lamellae from existing ones provide the plastids with a self continuity. The genes for the *de novo* formation of the layers as well as the possible differentiation of the plastids from cytoplasmic structures come to be superfluous and may be lost or changed to other functions during evolution. In this case the enzymatic formation of chlorophyll and other conditions for the maintenance of the chloroplast lamellae and layers in the dark are indispensable. This means that the layered chloroplasts have become autonomous and can be formed only from preexisting chloroplasts. Obviously no special hereditary factors in the layered chloroplasts are necessary for this; the formation of new layers and lamellae can still satisfactorily be controlled from nuclear genes. Certainly this evolutionary stage has been reached in many thallophytes. Against the widespread idea (e. g. GEITLER, 1955) that the development of chloroplasts from undifferentiated organells or structures is a phylogenetic reduction, stands the opposite conception, i. e. the exclusive autonomy of the plastids as found especially in thallophytes may be acquired during evolution.

The development of the chloroplast in barley is schematically summarized in Fig. 2 by 8 stages. The earliest proplastid stages analyzed in the leaf meristems are undifferentiated and do not contain — as far as can be seen in the electron microscope — other structural elements than the surrounding cytoplasm (cf. stage 1). In stage 2 the plastids increase in size, take an amöboidic shape and the plastid center with its tubular structure is formed. In the next stage (3) starch grains appear in the light region surrounding the plastid center. From the plastid center the material for the formation of the layers protrudes radially (4). The starch grains have broken down again. Within the protruded layers the first pieces of lamellae are formed locally and at mutually independent points (5). These pieces of lamellae multiply intensively by thickening and splitting.

It is important to notice that during the whole development no free ends of lamellae can be observed, and they seem to be connected always in pairs. The multiplication of the short lamellae proceeds very rapidly and in different order at the same time. In stage 4 and 5 the plastid center displays a transformation of its structure and is incorporated into the layer resp. lamellar system. It is in this connection that the center can take a crystalline appearance (e. g. LEYON, 1956). The con-

tinuous lamellar structure of the chloroplast is accomplished by the fusion of the short double lamellae as shown in stage 6. This is possible by the growth of the lamellae in the direction of their plane. The result of these two processes is a plastid with continuously transversed double lamellae (7). Then the differentiation of the grana structure as the last step to the fully differentiated chloroplast (8) is performed by one further splitting and by the incorporation of material as traced by the higher contrast in the electron microscope. From stage 4 to 8 the globuli are present in some clusters. They have, as shown from a mutant analysis (v. WETTSTEIN, 1954, 1957), a certain not yet explained function in the formation of the lamellae and grana structure. They contain pigments. They appear first within and around the plastid center.

The development is not synchronized for all chloroplasts in the same cell. One can find in young leaves all stages from 1 to 5 within a single cell. In somewhat older tissues the cells contain stage 5 to 8 together. Even cells with all developmental stages can be found. Furthermore the different phases of the formation of layers and lamellae can be found simultaneously in different regions of the same chloroplast.

It is not clear how the first double lamellae leading to stage 5 are differentiated, although a hypothesis that this is accomplished by the stretching and fusion of vesicles from the plastid center has been advocated for maize (HODGE, MC LEAN and MERCER, 1956). The formation of a continuous lamellar structure by fusion of short double lamellae has been found independently for the maize chloroplast and seems to be a general mechanism for morphogenesis of layered structures in the cytoplasm (HODGE, 1956) and the retinal rods of the eye (DE ROBERTIS, 1956).

In maize the development of etiolated chloroplasts has been studied. It is shown that the plastid center can be formed in the dark and can reach large dimensions, but since no chlorophyll is formed, neither layers nor lamellae are present. The globuli are completely absent in etiolated plastids. However, a short exposure to light, which results in the transformation of protochlorophyll to chlorophyll, gives rise to the globuli. In fact they seem to be the first newly formed structures in etiolated plastids. This is in harmony with the idea that chlorophyll is localized in the globuli (v. WETTSTEIN, 1957) and means that chlorophyll formation at least starts in developmental stage 4 of Fig. 2.

The background for discussing the chloroplast development in dif-

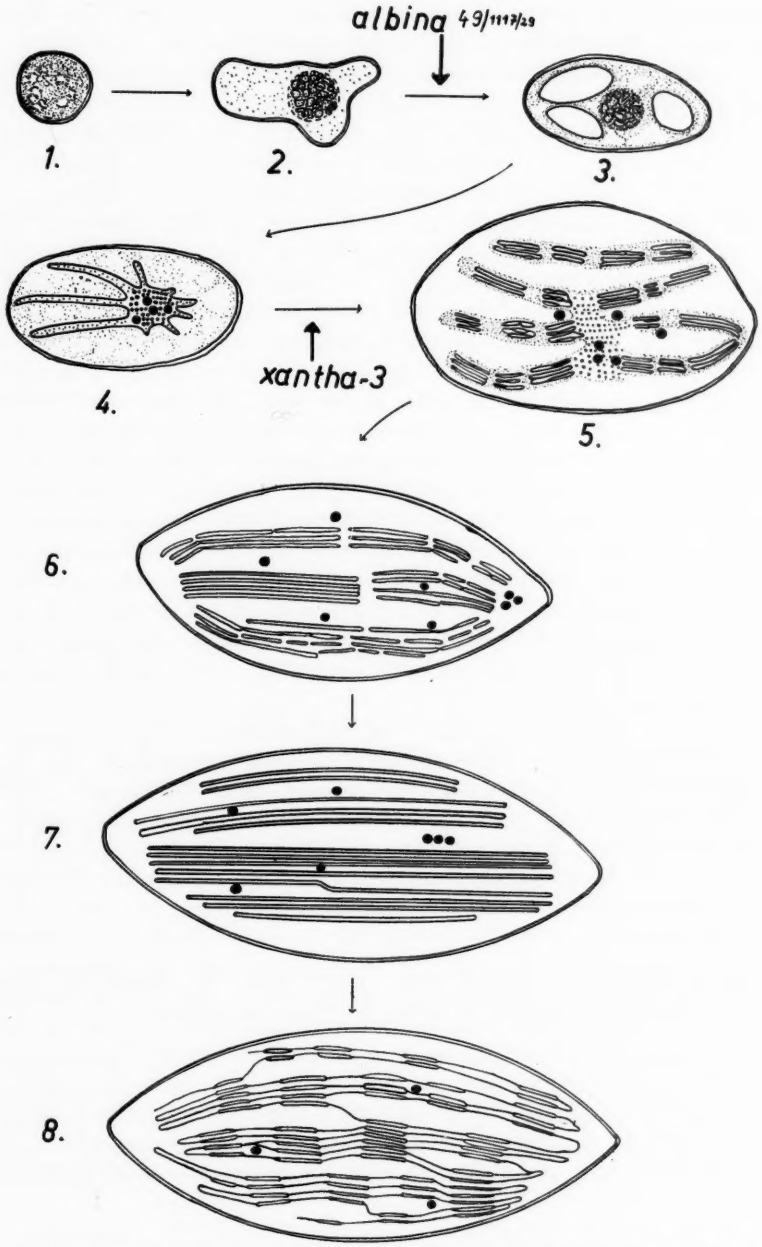

Fig. 2. Chloroplast-development and morphogenesis of the laminar organization in chloroplasts. For information see text.

ferent species is still scarce but some remarks can be made. According to LEYON (1956) the formation of the lamellar structure in *Aspidistra* is slightly different. The growth of the small double lamellae and the following fusion process seem to set in earlier. The multiplication of the lamellae takes place mainly in a stage with the established troughout lamellation (no 7 in Fig. 2), whereas in barley the multiplication of lamellae is mainly restricted to stage 5. We may now understand as well the structure of the *Oenothera* chloroplast (STUBBE and v. WETTSTEIN, 1955) in Fig. 1. Here in each layer only two or three of the short double lamellae grow and fuse, whereas the rest remain separated short double lamellae. In this stage the grana differentiation appears, thus before a complete throughout lamellation is established. In tomato (LEFORT to be published) the same situation is prevalent. The stage no 5 of Fig. 2 exists in young cells and then turns to a similar structure as for *Oenothera* in Fig. 1. The grana are differentiated and the chloroplast is able to form assimilates with this structural differentiation. In later stages a throughout lamellation is established. Thus the grana differentiation precedes here the formation of a completely connected lamellar system. This reveals some independence of the formation of the grana structure from the development of the general lamellar system. It will be of interest to know how far these variations of the same processes in morphogenesis of the submicroscopic chloroplast structure in different species can be changed by environmental factors and gene mutations.

Summing up the facts obtained in the analyses of chloroplast development, there is no evidence that the layers or lamellae or grana constitute units with hereditary independent continuity in spite of the fact that lamellae and layers can multiply from one another by thickening and splitting. The complicated lamellate structure of the chloroplast can be built up from structural elements not notably different from those found in the ground cytoplasm.

THE EFFECT OF GENOM AND PLASTIDOM MUTANTS ON THE SUBMICROSCOPIC STRUCTURE OF PLASTIDS

As indicated in Fig. 2 gene mutations causing chlorophyll lethals imply blocks in the development of the submicroscopic plastid structure at a certain point (v. WETTSTEIN, 1954, 1957). In the *albina* mutant 49/1117/29 the differentiation is blocked between developmental stage 2 and 3 of Fig. 2. It is a step of the inner structural differentiation of

the chloroplast which is prevented by the gene change. The plastid as a whole, however, continues to grow reaching nearly the size of a mature chloroplast although the inner structure remains the same as in stage 1 and 2 of the developmental chain. Thus the growth of the entire chloroplast is rather independent of its inner submicroscopic differentiation. The same applies to *xantha*—3. It will be an important task to analyze wether specific factor-mutations can change the growth of the entire chloroplasts without affecting their inner differentiation. The mutant factor *xantha*-3 gives rise to a block in a later stage of development, as indicated in Fig. 2. This mutant is of interest in so far as it shows that such chlorophyll lethals in higher plants can be of the same nature as the nutritional mutants studied so extensively in *Neurospora* and other microorganisms. *Xantha*—3 accumulates as a consequence of the gene change large amounts of globuli which may fill up the chloroplast entirely. Pigment analyses disclose that, together with the accumulation of the globuli, pigments and especially chlorophyll *b* are accumulated. This strongly suggests that chlorophyll and probably all pigments are localized in the globuli. The submicroscopic cytologist will thus be able to use factor mutations for the localization of pigment and other substances in specific submicroscopic structures. *Xantha*—3 shows another situation generally known from biochemical genetics. The accumulated globuli are broken down under the further growth of the plastids. At the same time the pigment content is diminished far below that of fully differentiated chloroplasts. This explains why an accumulation of the structural and chemical precursors to the synthetic step which is blocked cannot be caught in all such defect mutants. As soon as the rate of the degradation of the precursors approaches or reaches the rate of its production no accumulated intermediate can be found. These rates of degradation are certainly influenced by outer and inner environmental factors.

That this general conception of the chlorophyll lethals, caused by mutations in the genom, seems right, is supported by the recent study of factor mutations in tomato (LEFORT to be published). One of the mutants has its block approximately between developmental stage 3 and 4 of Fig. 2. Another shows its block just as *xantha*-3 between stage 4 and 5. However, there is no accumulation of globuli and the development exceeds that of *xantha*-3 reaching nearly stage 5. Without going into details the factor mutants will be of invaluable help in clarifying the processes of morphogenesis which are taking place between those major stages given in Fig. 2.

Mutants, affecting the developmental processes between stage 6—8, will certainly be found and should be looked for especially in the group of lethal *viridis* mutants. In general the viable *viridis* types probably reach stage 8, as shown by one analyzed mutant in maize (HODGE, 1956). As regards these viable *viridis* mutants it will be rather difficult to assert wether they primarily affect the plastids or, wether the effect on the pigments and plastids is secondarily brought about by some changes, for instance in the cytoplasm. The *albina* mutants on the other end of the chain will be of special help concerning the origin of the plastids. To drive it to an extreme: If proplastids can be condensed from cytoplasmic structures we should expect mutants in which no new plastids are formed besides those possibly esisting already in the egg cell, as far as such mutants are able to survive to the seedling stage.

Summing up, a great number of genes specifically affect the submicroscopic differentiation of the plastids. However, factor mutations which give rise to chlorophyll lethals are not necessarily connected with a primary block in chlorophyll synthesis.

The plastidom mutants investigated in *Oenothera* (STUBBE and v. WETTSTEIN, 1955) comprise two mutants of the *suaveolens* plastidom which give rise to chlorophyll deficient plastids in whatever genom combinatiom they are brought. The main result is that the chloroplasts of the »Yellow-green» and the »white» mutant type can perform a normal development and reach a differentiated submicroscopic structure, characteristic of a mature chloroplast. Only when this stage has been reached, a vacuolization of the plastids and a sort of hypertrophy of the peristromium sets in, earlier or later in ontogenesis and leads to a complete degeneration. The plastidom of at least these two types does not affect the development of the chloroplast. Its effect does not set in until the submicroscopic structure of the chloroplast is completed, i. e. when the chloroplasts are ready to start assimilation. I have tried to show (v. WETTSTEIN, 1957) that light microscopical studies of other »plastidom mutants» indicate a similar behaviour. So we arrive for the moment at the conclusion, that the plastidom does not control the development of the chloroplasts, which is contrary to what is generally assumed when said »these plastidom mutants have lost the ability for greening under all genom combinations». It is necessary to ascertain this principal difference between gene and plastidom mutations before any definite conclusions can be reached. The submicroscopic cytology of plastids provides a valuable tool for separating the action of the genom, plasmon, and plastidom.

This discussion may be rounded up by stating what really is established regarding non mendelian inheritance of plastid characters and what the assumptions are to explain the facts. For a broader discussion of the previous and new literature on the subject I refer to GUSTAFSSON and v. WETTSTEIN (1956).

The investigations of BAUR, CORRENS and RENNER have proved that defects on plastids can be inherited by an element outside the nucleus. They have further shown that this element in some cases is inherited only through the egg cell and in others both through the egg cell and the pollen. The extranuclearly inherited element in *Oenothera* has been termed plastidom by RENNER who has shown it to be very constant through many generations. SCHWEMMLE has provided data, which seem to prove that in *Oenothera* besides a plastidom also a plasmon can be demonstrated, so that two independent hereditary systems outside the nucleus are present. Furthermore he has shown that the plastidom even influences other traits than those of plastids.

It is also clear that the variegation is the result of a separation of the two plastidoms present in the fertilized egg cell during ontogenesis. The type of variegation is closely connected with the developmental differentiation of the leaf and stem tissues in the different species (RENNER, 1936 a, b; CORRENS and F. v. WETTSTEIN, 1937).

These facts are explained with the aid of the following assumptions. BAUR (1909, 1910) and RENNER (1922, 1936 and lit. cit. there) localize the plastidom in the plastids themself. This has been critisized by CORRENS and NOACK (see CORRENS and F. v. WETTSTEIN, 1937) and recently by MICHAELIS (1955, a, b) on various grounds. These authors consider a localization in the cytoplasm including other organells than plastids. The localization of the plastidom in the plastids is the background to the ideas of the segregation of different sorts of plastids during ontogenesis and the intensive search for the so-called mixed cells. Such an idea further rests on the assumption that the plastids are exclusively autonomous self duplicating organells. This is not yet conclusively established as pointed out repeatedly above. Although leucoplasts have been found in egg cells and pollen tubes, this does not mean that they give rise to all chloroplasts in the plant developing from such egg cells. The question of the mixed cell is a delicate one, since different developmental stages of plastids in normal plants are found within one cell and different degeneration stages are found in cells with defective plastids.

That the plastidom contains subunits may be indicated by the compe-

tition phenomena in *Oenothera* (RENNER 1924, SCHÖTZ 1954). Even these can readily be explained by a localization of the plastidom in the plasma, if we just consider the populations of different mutants of the k-particle in the cytoplasm of *Paramœcium* as a model (cf. GUSTAFSSON and v. WETTSTEIN 1956). If the plastidom comprises subunits of different quality we must ask if these act in one cell separately or as one entity and, in the latter case, if a single action depends on the numeric relationships between the qualitatively different subunits. In the case, that only a single alternative action on all plastids of one cell is possible, no mixed cells are to be expected even if the plastidom should be localized in the plastids themselves.

We have to close with the above mentioned problems and leave them open for the present. The submicroscopic cytology of plastids, however, will help us to solve at least some of the questions in continued studies.

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EFFECT OF SELECTION FOR MEIOTIC BEHAVIOUR IN AUTOTETRAPLOID RYE

By *GISELA HILPERT*
CYTOGENETIC DEPARTMENT, SVALÖF, SWEDEN
(Received November 23rd, 1956).

FOR many years tetraploid rye has been of great interest to breeders in several countries. However, in all induced autotetraploids fertility is more or less decreased as compared to the corresponding diploid material. In spite of the large grain size, good baking qualities such as high protein, large bread volume (MÜNTZING, 1951), possibly a lower diastatic activity (HINTZER and MIRANDA, 1954), the yield and straw-stiffness quite comparable to the best of the diploid varieties, so far no variety of »tetra rye» has been of real importance in practical farming. Higher yield, shorter and still stiffer straw and still better quality characters are required.

Increased yield may be obtained by increasing fertility and tillering capacity and some other characters, and intensive selection for higher fertility seems to be somewhat successful. As a rule good fertility seems to be correlated with regular meiosis (PLARRE, 1954 and BREMER and BREMER-REINDERS, 1954). Consequently intense selection of plants having a regular meiosis should be effective in producing »tetra»-populations with higher fertility. However, some authors (*e. g.* MORRISON, 1956) are of another opinion.

For some years one of the projects in the cytogenetic department at Svalöf has been to investigate the effects of selection for meiotic behaviour in the tetra rye as to changes in fertility. It is well known that at meiosis in an autotetraploid the chromosomes besides bivalents also form tetravalents, trivalents and univalents. Among these mainly the trivalents and univalents but also to some extent the straight ring tetravalents cause the observed meiotic irregularities and are responsible for at least part of the decrease in fertility and also the occurrence of the aneuploids. The problem of aneuploids will be delt with in a later paper.

To avoid winter killing and disturbances during the winter, spring rye was chosen as the material for this study, making the assumption

that conditions found in the spring rye may also to some extent be valid for winter rye. Among others the variety Sv 0201, a hybrid between the varieties Petkus and Od, was treated with colchicine in 1950. During 1953 plants of the C_4 generation were fixed in acetic-alcohol (1:3) for studies of meiosis. Owing to selection in the earlier generations the material could be divided into three groups: 1. unselected material, 2. material selected during three generations for high fertility, 3. material selected during three generations for high tillering capacity. The cytological investigation of this material was carried out during the winter 1954—1955 and according to the results the material was grouped in three classes A, B and C of equal size; A being the group with regular meiosis, C the irregular one and B the intermediate group. In the summer 1955 the three groups were grown separately and fixations made in acetic-alcohol (1:3). Meiosis was studied in squash slides and the chromosomes were stained with orcein. In the 1953 material the groups 1, 2 and 3 were represented by 38 different plants (15+16+7). The number of pollen mother cells (PMC) studied at metaphase I and possible to analyse completely was about 5—10 percent of all PMC observed. The total number of completely analysable cells was 859 (169+343+347), which means an average of 11, 21 and 38 cells, per plant respectively. In 1955 the total number of plants was 64, comprising 26, 21 and 17 individuals in the three groups A, B and C. The total number of PMC analysed at first metaphase was 1092 (331+337+424), which corresponds to an average of 24, 16 and 25 cells per plant studied.

For each cell the number of bivalents (BV), of zigzag tetravalents (ZZ), of straight ring tetravalents (RT), of trivalents and univalents, (T+U) and univalents (UNI) was scored. Mean values based on the number of individual chromosomes included in the different configurations have been calculated for the different groups and are given in Tables 1 and 2.

In the ZZ class all tetravalents with zigzag orientation rings as well as chains are included. The RT group includes all straight rings and chains, that are not completely zigzag orientated. The »ZZ-valents» always give a 2 — 2 distribution while »RT-valents» sometimes may, sometimes fail and instead give a 3 — 1 distribution or lose a lagging chromosome. Trivalents and univalents will normally give irregular distribution of the chromosomes to the daugther cells, and frequently laggards are found giving dividing single chromosomes at first anaphase which may be eliminated in the second division. Thus, it seems

TABLE 1. *Configurations at first metaphase of autotetraploid spring rye (1953).*

Number of chromosomes involved in	*BV*	*ZZ*	*RT*	*T+U*	*Uni*	Total
1. Population without any selection	18.9	1.8	4.4	1.9	1.1	28.1
2. Population selected for high seed set	22.1	1.9	3.1	0.6	0.3	28.0
3. Population selected for good tillering	23.0	1.7	2.6	0.4	0.3	28.0

quite logical to combine the BV and ZZ groups as the *regular* ones and RT and the others as *irregular* ones. MORRISON (1956) writes: »As it is obviously normal for an autotetraploid to have some quadrivalents what is needed is an abnormal meiosis with 14 pairs, or else 7 quadrivalents that will always separate equally». It is true that it is an abnormal meiosis but it is a regular one as the division and the distribution of chromosomes is regular. PLARRE (1954) classified all cells with multivalent configurations at first metaphase as aberrant PMC and only ring bivalents and rod bivalents were considered to represent the normal type of pairing.

In Table 1 there is shown a higher frequency of bivalent formation in series 2 and 3 than in series 1 representing the unselected material (quotient=27.96^{***} and $P < 0.001$). However there is no significant difference between 2 and 3. Possibly the selection in group 3 for high tillering capacity has resulted in selection for strong and healthy plants genetically and cytologically balanced euploids and, thus, automatically showing a good fertility and fairly regular meiosis. If the zigzag tetravalents are added to the bivalents there is the same difference between 2 and 3 on one hand and series 1 on the other. (Quotient=22.72^{***} and $P<0.001$). Still there is no significant difference between 2 and 3.

Further, there is no significant difference between the groups in the

TABLE 2. *Configurations at first metaphase of autotetraploid spring rye (1955).*

Number of chromosomes involved in	*BV*	*ZZ*	*RT*	*T+U*	*Uni*	Total
A. Population selected for regular meiosis	23.0	2.2	2.0	0.3	0.4	27.9
B. Population selected for intermediate meiosis	22.8	2.6	1.9	0.2	0.3	27.8
C. Population selected for irregular meiosis	22.8	1.8	2.6	0.5	0.3	28.0

orientation of tetravalents, *e. g.* zigzag contra open ring tetravalents. However, there seems to be fewer tetravalents in groups 2 and 3 than in group 1 (quotient=5.28*). The sum of chromosomes is deviating from 28 because of the aneuploids occuring in the material.

The material from 1955 was divided into three groups A, B and C according to the degree of meiotic regularity in the mother plants and is presented in Table 2. There are no significant differences in frequency of bivalents between the groups. If the ZZ class is added to the BV, there is a tendency towards higher means for A+B as compared to C (quotient=5.65*). The frequency of ZZ is slightly higher in A+B (quotient=4.94*). However, the total amount of tetravalents is not significantly changed.

In all groups of material more or less frequent irregularities were observed. Individual cells with 14 bivalents occurred but a whole plant with only this type of cell was not found. The average frequency of multivalents in this material was considerably lower than in MÜNTZINGs (1951) material, about 5.0 chromosomes per cell being included in multivalents, which corresponds to 1.25 tetravalents per cell. MÜNTZING found 3.95 multivalents per cell. However, he assumes that the frequency is still higher since cells with multivalents are more difficult to analyse. In the present material it has been possible to analyse completely only 5 — 10 per cent of all PMC at first metaphase. Thus, the true mean value may be considerably higher. However, PLARRE (1954) and BREMER and BREMER-REINDERS (1954) have foud a lower frequency of multivalents while MORRISON (1956) has found about the same high frequency as MÜNTZING. There may be some differences in multivalent formation between genetically different tetraploid rye populations, the present material being a spring rye rather distantly related to the winter rye earlier described.

It is surprising that the population selected for irregular meiosis has as low a frequency of multivalents as the positively selected group. Evidently one generation of selection for regular meiosis had no significant effect. However, there seems to be a tendency towards increased frequency of zigzag tetravalents.

MÜNTZING (1951) did not find any correlation between good fertility and regularity of meiosis in his winter rye material. Neither did ARMSTRONG and ROBERTSON (1956) find any correlation of this kind in tetraploid clover. However, BREMER and BREMER-REINDERS (1954) found a positive effect on regularity of meiosis by recurrent selection of fertile plants during 7 generations in their material of winter rye.

From the present investigation it is evident that not only a strong selection for high seed set but also a selection for tillering, *e. g.* strong and generally healthy plants, during three generations has resulted in a significant increase in meiotic regularity. So far it is also evident, however, that a single separate selection for regular meiosis has given no significant effect although there is a tendency in this direction. Continued experiments may give a more definite answer to the problem.

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THE INDUCTION OF VEGETATIVE MUTATIONS IN *RIBES NIGRUM*

By *RUDOLF BAUER*

MAX-PLANCK-INSTITUT FÜR ZÜCHTUNGSFORSCHUNG KÖLN—VOGELSANG

(Lecture delivered at the Fifth Radiobiological Conference in Stockholm, August 19th, 1956, Session on the use of Mutations in Plant Breeding)

I. INTRODUCTION

THE following report is based on investigations carried out since 1948 in the Max-Planck-Institut für Züchtungsforschung, Division for Small Fruit Breeding. They were suggested by KAPLAN (1953) and by Prof. Dr. W. RUDORF to explore the use of acute irradiation by X-rays as a method in practical breeding work with black currants.

Experiments in breeding black currants during a period of 14 years by TYDEMAN (1930, 1938) showed little promise of any improvements on the basis of seedlings, obtained from the chief varieties (Goliath, Boskoop Giant, Baldwin), neither by selfing nor by crossing.

On the basis of these results TYDEMAN concluded that outstanding seedlings, combining characters till now realised only in different varieties, may only be raised in very large families from a few selected first crosses and by intercrossing the best of these seedlings. But in order to achieve any great improvement in such characters as raceme length or fruit size beyond what is at present found in the best varieties, two other lines of future work must be employed:

1. hybridization with other related species,
2. use of methods promoting the occurrence of mutations.

On examaining these methods of breeding work on a small basis, TYDEMAN and coworkers (1938) obtained an outlook not particularly hopeful. They succeeded in crosses with *R. longeracemosum*, a species with the longest racemes of all known species of the genus *Ribes*, but they did not succeed in overcoming the sterility of these hybrids. Also their attempts to induce mutations in black currant seedlings by treating seeds, pollen and ovules with X-rays proved to be completely ineffectual. However, experiments carried out by us on a wider basis of

material and techniques employed confirm the opinion first pointed out by TYDEMAN.

In respect of the possibilities offered by hybridization with outstanding species, I succeeded in obtaining improvements in resistance to fungi (*Pseudopeziza ribis*, *Cronartium ribicola*) and to winterfrost in different combinations, as pointed out in a paper presented to the Horticultural Congress at Scheveningen 1955 (in press).

As regards the effectivity of X-rays, we also obtained promising results on the basis of vegetative mutations, induced by irradiation of wooded cuttings, but only in the cases of special arrangements during the years after irradiation. The principles of these methods worked out here may be employed for most crop plants, which are propagated vegetatively by shoots, rootstocks, and so on, and in which irradiations by X-rays had, so far, given poor results.

In the following the development of these methods for the isolation and separation of vegetative mutations will be demonstrated by the results of one of the main tests. This was made to find out the mode of action of X-rays at a rate of 3000 r, given in spring to wooded cuttings in a physiological state of activity.

II. MATERIAL AND METHODS

A. Material

To obtain a general view, the main test included a wide range of varieties of black currants in different provenances, which according to their relationship may be grouped in the following way:

1. Goliath-group:	4 varieties (6 provenances): Goliath (2), Invincible Giant (1), »Lissawaya» (2), Westwick Triumph (1)
2. Black-French-group:	3 varieties (5 provenances): Black French (1), Lees Black (2), Black Naples (2)
3. Silvergieter-group:	1 variety (4 provenances): Silvergieter (4)
4. Boskoop Giant-group:	2 varieties (5 provenances): Boskoop Giant (2), Rosenthals (3).

Shoots were taken from two-year-old plants when they became dormant (1.10.48). They were cut at a length of 15 cm with a well-developed terminal bud. These cuttings were put into moist layers filled with peat, where they wintered without frostprotection and from where

they were only removed for the time of irradiation. In early spring, before the development of buds and roots started, the cuttings were put into nursery-rows in a field with well-prepared peaty soil.

B. Dates of irradiation

The lethal dose of X-rays, concerning rooting and vitality of the cuttings, appeared to be 4000—6000 r after preliminary trials started in March 1948 by Prof. RUDORF, Dr. KAPLAN and Miss KRUMME. The dose-rate of 6000 r did not allow of any development, and the dose-rate of 4000 r checked the rooting in a high degree and the spring-off of the buds too. Thus, a dose-rate of 3000 r was chosen for the main test (April 1949). The material was ranged in layers of 2—3 cm under the X-ray-source (130 KVP. filter 0,5 mm Al.-equivalent, 2 mA, focus-distance 30 cm). To prevent the rooting capacity being influenced, the lower parts of the cuttings were covered with a sheet of lead (3 mm).

C. Way of observation and selection

The observations and the technical arrangements for the selection of artificial sports of the main test were based on observations of the surviving plants of the previous tests (March 1948), which were taken up by the referent since June 1948. In temporal range of development the following facts could be observed during the growth of the primary shoot, coming out from the X-radiated cutting:

1. Symptoms of mosaic in the leaves first developed, which decrease in number from the first to the seventh leaf but increase in size of chlorotic or necrotic spots. These may be explained as somatic chromosomal mutations and their intra-individual selection in the embryonic state of leaves irradiated in the bud (KAPLAN 1953).

2. As a rule, the immediately following leaves normalise and regain the specific type of the variety. Only very seldom are there shoots which show deviations in parts of the leaves (sectors) or changes in the arrangement of the leaves on the shoot: two leaves at one stalk or double buds in the same node, and more frequently a deranging of the whorl, which normally should be five-whorled.

3. In a higher degree there exist irregularities in the permanence of development at the top of the shoot. Sometimes it finishes its growth without any visible reason but some weeks later a new growth is started from the bud of the lower leaf.

4. In one case such a lateral shoot formed an anomalous type of foliation in which all developed leaves show the same types of deviation. This fact may be interpreted as a pointer to the origin of artificial sports.

With regard to the further behaviour of the terminal and lateral shoots in flower and fruit-set these observations were continued in the next season and gave the following results:

5. Most of the buds show a normal development. Only a small part remains without shooting forth.

6. As a rule the lateral shoots have normal leaves, typical of the variety. Very seldom, however, do shoots arise in which the whole foliage is changed in the same way. They mostly originate in the lower parts of the two-year-old primary shoots.

7. The number of flower buds of the primary shoot does not reach the controls. The clusters may vary in their length as well as in the number of flowers in the different regions of the shoot.

8. The fertility differs in a wide range from bud to bud on the same shoot as well as from flower to flower in the same cluster. The fruit-set is very irregular (0—normal) and the size of berries differs in a very high degree and not according to the normal order.

9. Measurements of the sizes of pollen grains and germination-tests show a remarkable frequency of disturbance in their form and physiological state. Thus, the sterility of flowers and the development of small-sized berries with only a few seeds may be explained by disturbances in the meiosis which begin with the anthers in autumn but, as regards the egg-cells, not until the following spring. Consequently, these facts cannot be explained as a direct physiological reaction to the irradiation but as a change of the genetical structure in the chromosomal set.

Summarizing the observations of the first year, the effect of X-radiation generally seems to be restricted to overgoing »Primäreffekte», which do not influence the further development of the primary shoot. Only by exact observations do we notice in rare cases disturbances of parts of the shoots which may take part in the further development of the plant in the following year. Together with the results of the second year the observations point to a chimerical character of the primary shoots, in spite of their phaenotypically »normalized» appearance.

In regard to the frequency and localisation of such changed tissues we may suppose that during the period of vegetation intra-individual

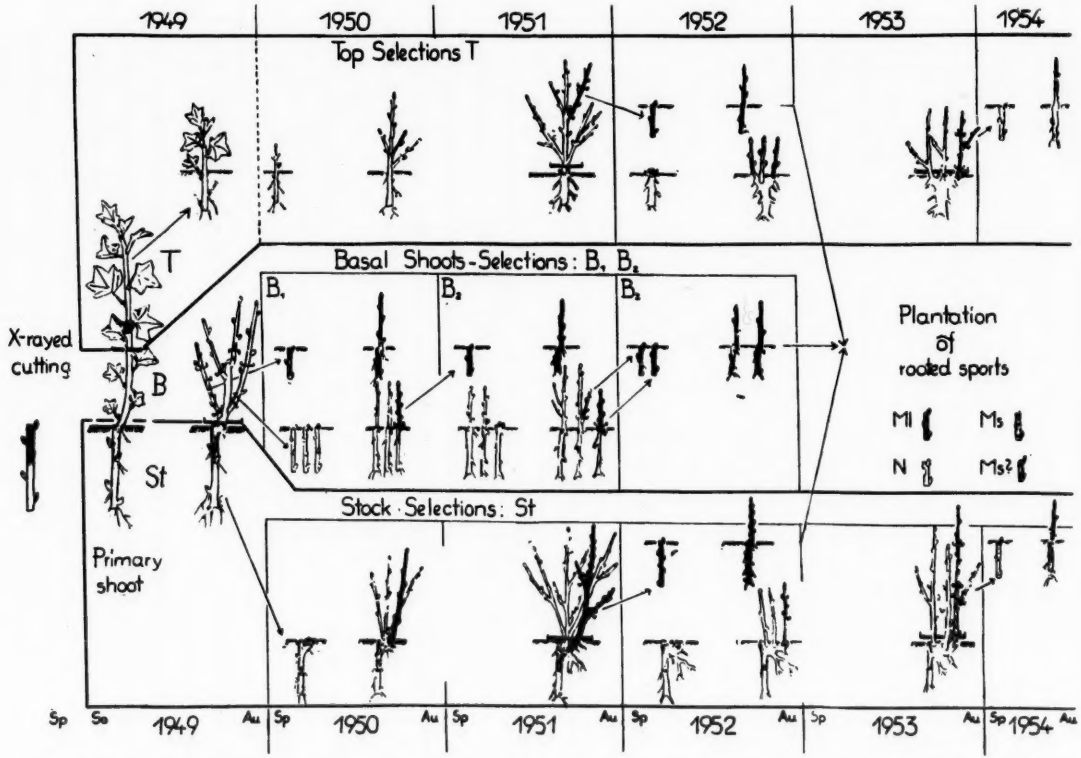

Fig. 1. Scheme of the method employed to determine the sporting capacity in the different parts of the plant grown up from the irradiated cutting:

T=decapitated top, B=basal region, St=rootstock of the primary shoot, ⌴=sign for cutting back

selections are at work by which normal tissues become more and more dominating (normalising-effect). Only a few parts of the changed tissues are able to build up buds which originate a phaenotypical appearance in the form of »artificial» sports. Most of the changed tissues remain without phaenotypical signs, especially in the basal parts of the primary shoots. Therefore, in the main tests the technical arrangement aimed at separating mutagenic-changed tissues by a furthering of bud-shooting in the lower parts of the primary shoot. The simplest way was to divide the primary shoot into small cuttings and after their rooting to select all arising artificial sports. To test the assumption of intra-individual selections between normal and changed tissues in the primary shoot, the following scheme was employed to determine the content of vegetative mutations in the top and basal parts of the primary shoot and the remaining rootstock. (Fig. 1).

D. Arrangements in the main trial during the first two years of development

I. The primary shoot which originates from the X-radiated terminal bud of the cutting must be cut after having grown to a length of 15 cm (Fig. 1, summer 1949). Only the basal region of the shoot, showing primary effect of mosaic, remains on the rooted cutting. By decapitating the shoot, the development of lateral shoots is induced in the remaining basal leafbuds: they develop till the end of the first year into woody shoots and among them the first selection of artificial sports can be performed (Fig. 1: B_1=Basal-selection).

The remaining »normal» shoots were divided into small cuttings, and after rooting they were tested during the following two years and the artificial sports were registered and selected (Basal-selection: Fig. 1: B_2). The occurrence of abnormal shoots in both selections indicates the presence of mutated tissues which have grown in the basal part of the primary shoot.

II. The removed tops were divided into smaller parts and rooted under glass (green-cuttings). Every part grew up to a plant in which artificial sports may be observed. Its selection cannot be realised before these plants grow up to branched shrubs (Top-selection: Fig. 1: T).

III. To control the further behaviour of the rooted cuttings in which the X-radiated terminal bud had grown up to a little stock-base, these stocks were planted out to regenerate their shoots. The selections which can be made among these regenerates indicate the rate of mutated tis-

sues in the base of the bud which was originally irradiated and had at first remained dormant (Stock-selection: Fig. 1: St).

E. Mode of selection

The selection of changed shoots was carried out in two ways:

1. Observation of deviating characters in the structure of the shoots and the foliage and registration of their frequency in relation to normal shoots grown on the same plant at the same time. This work was undertaken late in the season when shoot-growing was almost finished.

2. Registration of deviating characters in regard to the development of flowers and fruits and other practically important characters such as season of flowering and ripening, fertility, length and weigth of clusters, size and flavour of berries etc. These observations also included shoots with a normal foliage but an outstanding behaviour in one or more of the above mentioned characters. These selections are based on measurements and weighings.

The isolation of the registered sports was practised by cuttings which after rooting were tested for their further behaviour in the following years.

F. Arrangements during the following years

After the first selecting-period (1950—1951) the shrubs were cut back totally to the ground to control their further content of changed tissues (Fig. 1: autumn 1951).

After a second period of selection (spring 1952—autumn 1953) a second cutting back was employed.

G. Determination of the rate of mutation

To find a basis for the rate of mutation, two modes of calculation may be used:

1. Determination of the proportion between the number of X-radiated plants at the date of observation and the number of artificial sports which grew up in these plants.

2. Comparison between the number of normal shoots and the number of changed ones.

The difference obtained, when comparing both modes, results from the fact that as a rule one X-radiated stock may build up more than one shoot after having been cut back. These shoots can all be changed

— in the same way or in a different way — or only some of them become abnormal. The following data are based on the proportion between number of X-radiated plants and number of sports derived from them.

III. RESULTS

A. Rate of vegetative mutations

Summarizing all types of artificial sports which were selected from all irradiated cuttings (343) and their descendants during a period of five years (1949—1953) we got 324 different selections, that is to say, nearly every irradiated cutting develops one artificial sport on the average. The rate of vegetative mutations calculated in this way reaches nearly 100 %. This high efficiency of X-rays on buds in a post-dormant state is surprising. It results from the technique of dividing the primary shoot into smaller parts on the one hand and of cutting back the originally irradiated root-stock once or twice on the other hand. This rate of vegetative mutation is composed of the different selections from the primary shoot (top=T, basal=B_1, B_2) and from the root-stock (St.) in different years (Tab. 1).

About one sixth of all sports derives from the propagations of the upper parts of the primary shoots (T). Twice that number of sports was found in the branch shoots (B_1) and in the cuttings which were made of the remaining »normal« branch shoots from the basal part of the primary shoot (B_2). Half the number of all sports was gained by twice cutting back the root-stocks which were developed from the cuttings originally irradiated (St.). Comparing these proportions, four facts can be pointed out:

1. These results verify the assumption of the chimerical nature of the primary shoot as well as of the root-stock, built up by the bud of the cutting originally irradiated.

2. There are intra-individual selections between changed and unchanged tissues during the growing up of the primary shoot. The »normalising«effect may be explained by a higher rate of vitality in the unchanged parts of tissues.

3. The technical arrangements employed, aiming at a maximal developing and shooting of buds by a repeated cutting back, may be one of the most effective modes for splitting up the induced chimera in its components and for giving possibilities for its selection.

4. Comparing the amounts of artificial sports selected after the first

TABLE 1. *Rate of vegetative mutations in the different selections of the primary shoot and root stock from 1949 to 1953.*

Kind of selection	Date of selection 1949 Ms+1	1949 Ms?	1951 Ms+1	1951 Ms?	1953 Ms+1	1953 Ms?	1949–1953 Ms+1	1949–1953 Ms?	Sum of sports Ms+1+Ms?	Per cent	Number of observed cuttings
Primary shoot											
Upper part											
T-selection	—	—	18	3	19*	5	37	8	45	13.9	(160)
Lower part											
B1-selection	45°	12									343
B2-selection			44	10	—	—	89	22	111	34.2	(461)
Irradiated cutting											
St-selection	—	—	79*	27	54*	8	133	35	168	51.8	343
Sum of all years	45 + 12		141 + 40		73 + 13		259 + 65		324		343
	57		181		86		324				
	17.6 %		56.0 %		26.6 %		99.3 %			94.5	

*=after cutting back totally; °=after cutting back partially; ()=number of descendants; Ms=small mutation; Ml=large mutation; Ms?=›uncertain‹ small mutation.

TABLE 2. *Percentage of artificial sports.*

Variety and provenance		Number of irradiated cuttings	Number of mutated descendants in the different selections:			Sum of mutations 1949—53	Per cent
			St.	B_1+B_2	T		
Goliath	b	25	23	11	1	35	140
Goliath	c	6	10	3	0	13	216
Invincible Giant	a	15	11	7	0	18	120
»Lissawaya»	b_1	9	3	2	2	7	78
(control)	b_2	7	3	3	0	6	86
Westwick Triumph	a	13	6	4	0	10	77
Goliath-group		75	56	30	3	89	119
Merveille de la Gironde	b	36	23	11	12	46	138
Lees Black	a_1	15	3	7	2	12	80
(control)	a_2	10	4	5	0	9	90
Black Naples	a	35	11	15	11	37	106
Black Naples	d	9	3	2	3	8	89
Black French group		105	44	40	28	112	107
Silvergieter	b	32	10	9	6	25	78
Silvergieter	T_1	8	10	5	1	16	200
Silvergieter	T_2	16	9	6	2	17	106
Silvergieter	T_3	19	9	4	0	13	68
Silvergieter-group		75	38	24	9	71	95
Rosenthals	c_3	10	1	3	0	4	40
Rosenthals	T_1	13	5	3	1	9	69
Rosenthals	T_2	10	3	0	1	4	40
Boskoop Giant	b_1	28	11	5	2	18	64
(control)	b_2	27	10	6	1	17	63
Boskoop Giant-group		88	30	17	5	52	59
Sum total		343	168	111	45	324	94.5

and the second cutting back we see a diminution of sporting capacity to half the amount in the season following the second cutting back.

The rates of sporting given in Table 1 are the summarized values of all tested varieties. Regarding the behaviour of each separate variety (Tab. 2) we find very high differences between them. As is seen, all provenances of »Boskoop Giant» (syn. »Rosenthals») show a small rate of mutability. In some provenances of the varieties of the other

Fig. 2. Type of »large» mutation, selected from X-radiated (3000 r) cutting of the variety of »Black Naples» in the first year of development (rooting-season). Characters: Lasciculated leaves, somewhat shortened internodes, increased development of the lateral shoots. In the following years totally sterile in selfing and crossing.

groups, the rate of mutability reaches an amount of more than 200 % (Silvergieter $T_1=200$ %, Goliath $c=216$ %). From practice we know that, for example, in comparing provenances of the variety of »Goliath» we may find obvious differences in one or more characters, due to a certain degree of natural sporting.

The differences in the capacity for an induced sporting are made sufficiently sure by controls in which the radiated cuttings derive from two different mother-plants of the same provenance and from which we got only differences of 1—10 % in the rate of sporting. (»Goliath» $b_1: b_2=8$ %, »Lees Black» $a_1: a_2=10$ %, »Boskoop Giant» $b_1: b_2=1$ %).

B. Characters of the artificial sports

Regarding the characters of the selected artificial sports and their value for practical use, only a very restricted general survey can be given here. A more detailed report will be published later on.

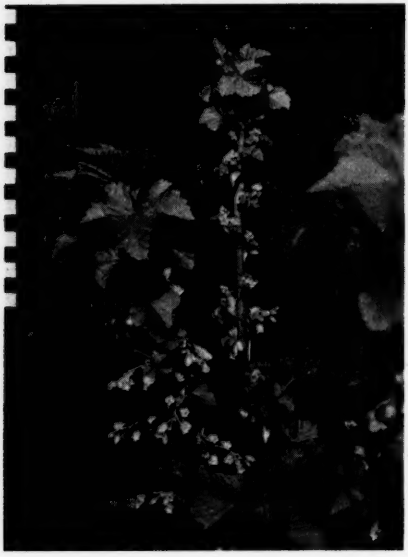

Fig. 3. Type of a »small» mutation with a changed flowering-time (8 days later), shortened internodes and smaller leaves (middle shoot). Photo is taken from the shrub in which this sport is developed during the previous season. Normal shoot on the left. Further characters: fruit ripeness four days later, fertile only after crossings not after selfings.

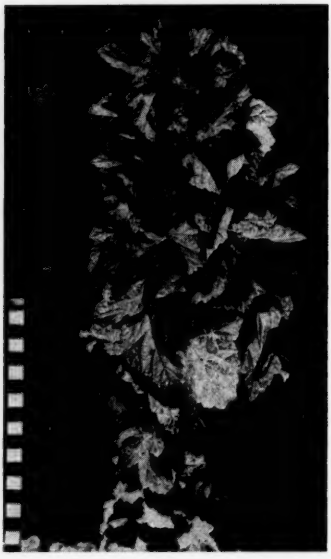

Fig. 4. Type of a »small» mutation, selected from X-radiated (3000 r) cutting of the variety of »Goliath» in the first year of development (rooting-season). Characters: Extremely shortened internodes, normal size of leaves; in the following years normal in fertility and normal size of clusters, but increased size of berries.

When registering the deviating shoots, three groups of sports may be distinguished:

1. »Large» mutations (Ml) in which the normal type is changed in many characters.

2. »Small» mutations (Ms), which only differ from the controls in a few details of shoot- and leaf-formation.

3. »Uncertain» small mutations (Ms?), which only differ from the controls by giving a higher yield.

The mutants of the first group may show extreme deviations regarding the type of foliage and structure of growth (Fig. 2). Most are totally sterile. The differences even reach the extent which is given between species of the genus *Ribes*.

In the sports of the second group, the principal characters of the variety can be recognized (Figs. 3, 4). These sports are fertile in crossings but their ability of selfing seems to be disturbed.

In the group of »uncertain» sports all types are summarized which cannot be differentiated from the controls by clear differences in leaf and shoot characters but by an outstanding behaviour in the fruiting characters of their shoots (average of weight and length of clusters, fruit size and quality of berries).

Regarding the frequency of these different types of sports it is evident from Table 3 that the highest frequency is represented by the »small» mutations (Ms=65 %). »Large» mutations and »uncertain» mutations take part only with an amount of 16 % and 20 %, respectively. The occurrence of these types is independent of the source of selection. We get the same proportions for these different types of sports in the three categories of descendants of the irradiated cutting (T, B_1, „ St.).

TABLE 3. *Frequency of »uncertain» small mutations (Ms?), small mutations (Ms), and large mutations (Ml).*

	Stock selections (St)	%	Basal part selections ($B_1 + B_2$)	%	Top selections (T)	%	Sum of all selections	%
Ms?	35	20.8	12 10	19.8	8	17.8	65	20.0
Ms	105	62.5	38 35	65.8	30	66.7	208	64.3
Ml	28	16.7	7 9	14.4	7	15.5	51	15.7
Total	168		57 54 111		45		324	

C. Practical value

All features which may be positive in regard to practical breeding work were collated. In the following the reported percentages represent the frequency of each feature in the total of all sports.

A higher vigour was realized in 9 %, a shortening of the internodes in 4.3 %. Both characters are combined only in some cases. This combination allows a higher yield on the same area.

In the fruiting characters there are types with elongated clusters in 4 %, larger weight of clusters in 9 %, and larger fruits in 5 %. In regard to »running off», positive selections could be made at a rate of 3 %, to a better flavour in 7 %, to a later ripening season in 1 %.

The susceptibility to *Cronartium ribicola* was changed into a moderate resistance only at a rate of 2 %.

The frequency of artificial sports in which only positive features are combined in one plant is very small. Up to the present we could get such selections in three cases only, that is to say, 1 %. This value is twice the rate of the outstanding selections we got from seedlings of the same varieties after selfing- and crossing-pollination during the same period.

It may be mentioned that most of the selected artificial sports combine both positive and negative characters, or include only negative characters. For instance, a reduction of fertility can be observed very frequently. Phenotypically this may be characterized by smaller berries, increased degree of »running off», or failing fruit-set after selfing and crossing.

Generalizing, we may say that »large» mutations are worthless in every case, but »small» mutations, especially sports which are designated as »uncertain» (Ms?) upon first observation, may represent improvements compared with the control. In total these results demonstrated that X-radiation can be a tool of breeding black currants. In this work the occurence of vegetative mutations by irradiation of wooded cuttings should be combined with a repeated cutting back in the following seasons of growth in order to utilize the capacity for sporting which remains in the X-radiated chimerical region of the developed root-stocks.

This method of breeding seems to be more effective than the line based on crosses between the best varieties. In contrast to the latter, its use can also be practised in large plantations, which are built up by shrubs grown up from irradiated cuttings. Besides the possibility of getting a nearly normal crop, changed shoots combining only positive characters can be selected. This also means a cheaper technique in the practical breeding work.

The application of this method may be useful for all those crop plants in which dormant buds build up a root-stock from which shoots are regenerating for several years and in which X-radiation tests so far seemed to be ineffectual or gave only small rates of sporting. Investigations by ZWINTSCHER (1955) based on the results of the tests reported above indicate how it may be applied to pome- and stone-fruits.

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INTERSPECIFIC CROSSES IN LUPINUS

CYTOLOGY AND INHERITANCE OF FLOWER COLOUR

By *MARIE BRAGDØ*

INSTITUTE OF GENETICS AND PLANT BREEDING, AGRICULTURAL COLLEGE OF NORWAY, VOLLEBEKK, NORWAY

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I. INTRODUCTION

A breeding programme with garden lupins was started in 1950 at the Agricultural College of Norway. The purpose was to produce valuable garden varieties, suitable for the conditions in Norway, and to study the cytology and genetics of some species and species crosses, especially with regard to flower colour. The work reported here was carried out partly at the Agricultural College of Norway, and partly at the John Innes Horticultural Institution, England.

The following species are included in the study:

Lupinus polyphyllus, LINDL.	$2n=48$ (TUSCHNJAKOWA, 1935).
Russell-lupin.	
Lupinus arboreus, SIMS.	$2n=48$ (SAVCHENKO, 1935).
» *nootkatensis*, DONN.	$2n=48$ (MAUDE, 1940).
» *luteus*, L.	$2n=46$ (DE ZEEUV, 1936)
	$2n=48$ (KAWAKAMI, 1930)
	$2n=52$ (TUSCHNJAKOWA, 1935).

L. arboreus, *L. polyphyllus* and *L. nootkatensis* are perennial species, natives of North America. *L. arboreus* has its distribution along the coast of California (JEPSON, 1911). *L. polyphyllus* is distributed from Washington to California (BAILEY and BAILEY, 1941) and according to JEPSON also in the interior and at higher altitudes. *L. nootkatensis* is distributed near the sea, from Washington to the Aleutian Islands (BAILEY and BAILEY, 1941). The *Russell-lupin* is of unknown origin, but it is supposed to be a hybrid between *L. arboreus* and *L. polyphyllus*. *L. luteus* is an annual species, a native of South Europe, distributed near the Mediterranean Sea. (FISCHER and SENGBUSCH, 1935).

L. polyphyllus and L. nootkatensis are hardy species, capable of

growing in most parts of Norway. But except for the hardiness they are of poorer quality than the *Russell-lupin*. The latter is, however, not well fitted for the winter in Norway.

Except for *L. arboreus*, the seed used was of commercial varieties. The seed was sown in a greenhouse. When sown early in spring, some of the plants gave ripe seed in the same year. The parent plants and the F_1 plants were kept in an insectproof greenhouse during the flowering period. The parent plants were emasculated and pollinated one day later. Due to low self-fertility, the F_2 generations were in most cases produced by intercrossing the F_1 plants, in some cases by selfing. The F_2 plants were transplanted to the field.

For the cytological study, the flower buds were fixed in summer in Carnoy's fixing fluid, and kept for some months in 70 % alcohol. The anthers were squashed and stained in aceto-carmine with iron.

The tests of the pigments were done by means of paper-chromatography (BATE-SMITH, 1949).

II. RESULTS

1. Fertility

Hybrids were obtained in the following cross combinations:

L. polyphyllus × *Russell-lupin*
» » × *L. arboreus*
» *nootkatensis* × *Russell-lupin*
» » × *L. arboreus*

Only a small per cent of the flowers pollinated set seed, but the low fertility may be due to damage when the flowers were emasculated, as they are very delicate at the stage when this is done.

The hybrids were fully fertile when they were cross-pollinated. The species as well as the hybrids set little seed after spontaneous selfing, though somewhat more when the flowers were tripped.

L. polyphyllus × *L. luteus*

No hybrids were obtained in this cross. Using *L. polyphyllus* as female 550 flowers were pollinated and gave no seeds. In the reciprocal cross 23 seeds were obtained from 450 flowers pollinated. They were, however, poorly developed and did not germinate. This cross, among others, involving Old-World-species, is also reported as infertile by GOLLMICK (1937).

2. Morphology

Table 1 gives a rough comparison between the species and the F_1 plants of some characters of interest from a horticultural point of view. Most of these characters show a great variation also within the species, and no exact measurements were made in this material. The *Russell-lupin*, especially, is very variable, and plants with low compact growth, compact spikes and large flowers selected from a commercial strain, were used in this work.

L. polyphyllus×*Russell-lupin.* In most of the characters mentioned, F_1 was intermediate between the parents, though there was a difference between different crosses, as would be expected from the variability in the *Russell-lupin.* There was also a marked difference between the F_2 families, some of them approaching one or the other of the parent types in appearance.

L. polyphyllus×*L. arboreus.* The F_1 plants were intermediate in most characters, and in the F_2 generation the habit as well as leaf size, length and compactness of the spike, showed a continuous range between the parent types.

L. nootkatensis×*L. arboreus.* Especially the young F_1 plants grew very much like *L. arboreus*, with one branched stem. The following year the growth habit was more like that of *L. nootkatensis* with many stems from the ground, but the plants grew much taller than *L. nootkatensis.* The F_2 generation showed a great variation with respect to habit, compactness of the spike, leaf size and leaf type. Most plants were found in the intermediate classes.

L. nootkatensis×*Russell-lupin.* As in the other crosses, the F_1 plants were intermediate between the parents, though some characters, such as size of leaves and flower and length of spike, mostly tended towards the Russell parents. The same was the case in F_2, where only a few plants had flowers as small as those of *L. nootkatensis*, and many of them had flowers as large as those of *Russell-lupin.* The stems of the F_1 as well as of the F_2 plants were often tall and shrubby, unlike either of the parents.

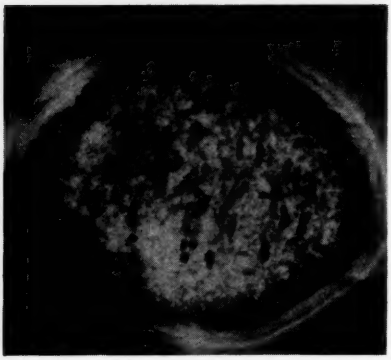
3. Cytology

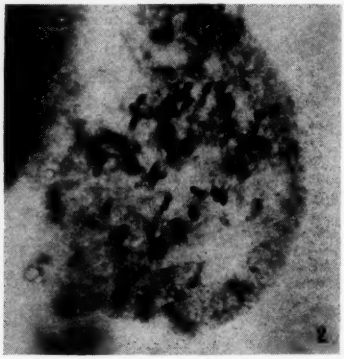
Meiosis was examined in the following material:

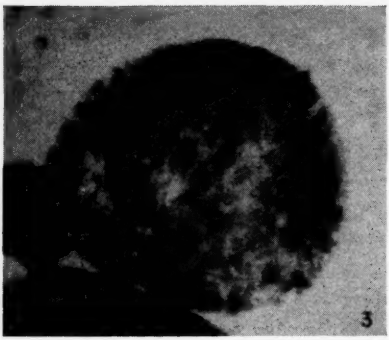
L. polyphyllus
Russell-lupin
L. arboreus
» *nootkatensis*

TABLE 1. *Comparison of F_1 and parent plants grown in pots.*

Species	Habit		Leaf size	Spike		Flower	
	1st year	2nd year		length	compactness	size	colour
L. pol.	1 or more stems little branched	more stems little branched	large	long	medium	medium	pink blue
F_1 *L. pol.* × *L. arb.*	1 stem branched	more stems little branched	medium-small	medium	medium	medium	light purple
L. arb.	1 stem much branched	1 main stem much branched	small	short	loose	small	yellow
F_1 *L. nootk.* × *L. arb.*	1 stem branched	more stems little branched	small	short	loose	small	light purple
L. nootk.	1 stem little branched	more stems little branched	medium	short	medium	small	blue
F_1 *L. nootk.* × *Russell*	1 shrubby stem little branched	more stems little branched	medium-large	medium	medium	medium-large	dark purple
Russell	1 or more stems branched	more stems branched	large	long	compact	large	yellow







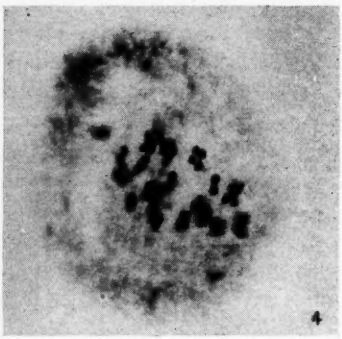


Fig. 1. First metaphase in *L. nootkatensis*×*L. arboreus*. — Fig. 2. First metaphase in *L. polyphyllus*×*Russell-lupin*. — Fig. 3. Second metaphase in *L. polyphyllus*. — Fig. 4. First metaphase in *L. polyphyllus*×*L. arboreus*.

L. polyphyllus ×*Russell-lupin*, F_1 *and* F_2
» » ×*L. arboreus*, » » »
» *nootkatensis*×*Russell-lupin*, » » »
» » ×*L. arboreus*, » » »
(» *polyphyllus* × » »)×*Russell-lupin*

Both in the species and in the hybrids 24 bivalents were found in all plants. Meiosis was regular in the hybrids as well as in the species. No univalents or multivalents were observed. (Figs. 1 and 2.) Owing to the small size of the chromosomes an exact estimation of the chiasma fre-

quency was not possible, but it seemed to be at least as high in the hybrids as in the species.

At second metaphase the chromosomes often lay together in pairs or in groups of three or more (Fig. 3). The same pairing was also observed at first metaphase, though not so frequently, (Fig. 4). Yet it may have been as common at first metaphase as at the second one, only not so easily seen, since the bivalents often were not so well spread out at first metaphase, and it was impossible to say if they lay in groups or not. Usually they did not touch one another, though sometimes they appeared to be connected.

This grouping or pairing was observed in the species and in all the hybrids. In some cases it may be due to stickiness, but the chromosomes seemed to contain much heterochromatin, and the reason may be a heterochromatic attraction, as shown in *Cicer arietinum* by THOMAS and REVELL (1946). The chromosomes in *Lupinus* were, however, too small to allow any closer investigation. The average length of a metaphase bivalent was about 3,5 μ. The same grouping or pairing has been observed in other Lupin species by SENN (1938).

4. Inheritance of flower colour

a. Introduction

To describe flower colour, which refers to the wings and the outer part of the standard, the colour chart Unesma 24 Farbentafeln was used. In this paper only the hue numbers are given, and the corresponding hue numbers of the Horticultural Colour Chart (H. C. C.) are also referred to. As the flower colour of some of the material showed a more or less continuous variation, the description was very difficult. However, the material could be separated into three distinct main groups, which will be dealt with in this paper. The following terms for colour are used:

		Unesma hue numbers	H. C. C. hue numbers
I. Acyanic, including	a. white		
	b. yellow	1,5— 2	2— 4
II. Cyanic			
1. reddish, including	a. orange	4 — 6	11—17
	b. red	6,5— 8	19—23
	c. pink	8,5— 9,5	24—28 (light tones)
2. bluish, including	a. purple	10 —11,5	30—36 (dark »)
	b. blue	12 —13	37—43

In the *Russell-lupin* all the colours mentioned occurred. In *L. polyphyllus* white, pink and blue types exist. Some variation occurs also in this species, but not as much as in the *Russell-lupin*. The colour of *L. arboreus* may be yellow, purple, or yellow mixed with purple. The types used in these crosses were yellow, but in some plants a weak tinge of purple appeared in old flowers. The flowers of *L. nootkatensis* are blue. and may, according to BAILEY and BAILEY (1941) also be variegated with red or yellow. Only the blue types were used in the crosses.

b. Inheritance in L. polyphyllus

The following crosses have been studied: a. blue×white, b. pink× white and c. blue×pink. The F_1 plants of all crosses were blue flowered. In the F_2 generation from the crosses a. and b. a ratio of 3 coloured (cyanic) : 1 white (acyanic) was obtained and in the cross c. all F_2 plants were coloured (Table 2). These results indicate a recessive gene *a*, giving white flowers. The coloured flowers from cross a. were all blue, while those from cross b. and c. showed a segregation of 3 blue : 1 pink. This indicates another recessive gene, *b*, which segregates independently of a, and converts the blue colour into pink. Thus *AB* plants are blue, *Ab* plants are pink, *aB* and *ab* plants are white.

The stems of plants with coloured flowers had more or less bluish colour, while those of white flowered plants were pure green. It seems therefore that *a* is a gene which suppresses the anthocyanin production, acting in the stems as well as in the flowers.

A corresponding anthocyanin suppressing gene was found in *L. angustifolius* by WESTERGAARD (1919) and by HALLQUIST (1921), though the suppression was not complete, as old flower of acyanic plants developed some colour. In this species HALLQUIST found two genes necessary to change the colour from blue to red.

c. Inheritance in the Russell-lupin

In Table 3 are given the results of the study of anthocyanin inheritance in this species. Eight crosses cyanic×acyanic gave in F_1 only plants with cyanic flowers. Four crosses gave a total offspring of 73 cyanic : 73 acyanic plants, showing that the cyanic parents were heterozygous for the anthocyanin gene. Selfing of the cyanic F_1 plants gave an F_2 consisting of 516 cyanic : 166 acyanic plants. Selfing of acyanic F_1 plants gave only acyanic offspring (122 F_2 plants) and the same result was obtained in intercrosses betwen acyanic plants (167 plants). The results show a clear-cut single gene segregation.

TABLE 2. *L. polyphyllus.*

Fam.	Parents	F_1	F_2(F_1 plants selfed or intercrossed)			
			cyanic			acyanic
			blue	pink	total	
82	white × blue	blue	65		65	19
83	blue × white	»	216		216	57
84	white × pink	»	271	75	346	105
85	pink × white	»	110	42	152	67
87	pink × blue	»	291	85	376	

Fam. 82, 83, 84, 85: Obtained 779 cyanic: 248 acyanic
Exp. 770,25 » : 256,75 » , $\chi^2 = 0{,}40$, $P = 0{,}5-0{,}8$.
Fam. 84, 85, 87 : Obtained 672 blue : 202 pink
Exp. 655,50 » : 218,50 » , $\chi^2 = 1{,}66$, $P = 0{,}1-0{,}2$.

Except for the families 608 and 609 all cyanic plants in these crosses belonged to the reddish group, showing that the acyanic parent plants did not carry the genes necessary for development of bluish colour, contrary to the acyanic plants in the *L. polyphyllus* crosses. These results were to be expected, as plants with bluish colour were scarce in the strain from which they were selected.

TABLE 3. *Russell-lupin, segregation of cyanic—acyanic.*

Fam.	Parents		F_1		F_2 from selfing cyanic F_1 plants	
	cyanic	acyanic	cyanic	acyanic	cyanic	acyanic
232	yellow	red	54		31	5
241	»	»	30		10	3
243	»	»	30		23	6
245	»	»	34		108	32
613	»	»	8		19	5
610	white	»	24		25	6
605	»	»	16		113	52
609	»	blue	2		20	4
235	yellow	red	32	37	55	17
239	»	»	34	30	61	15
606	white	»	2	2	15	7
608	»	blue	5	4	36	14
Total					516	166

In Table 4 are given data on the segregation of white—yellow flower colour in acyanic families and in the acyanic groups in families segregating also for anthocyanin. In the acyanic families, selfing and intercrossing of yellow plants have in 10 families given only yellow offspring (1. Table 4). Selfing and intercrosses of white plants have given either exclusively white offspring or segregation in white and yellow (2. Table 4). One family from the cross white—yellow has given only white offspring, while 12 families have given 114 white : 148 yellow. The data indicate the segregation of a dominant gene for white flower colour. There is a tendency to a surplus of the maternal flower colour, which may be due to the occurrence of some selfing, the plants in these crosses not being emasculated. In crosses under 4, 5 and 6 in Table 4 is given the segregation of yellow and white in the acyanic plants in crosses between cyanic and acyanic plants. The results agree with the hypothesis of a dominant gene for white—versus yellow, except in the case of 4 families giving 21 white : 133 yellow. If the red parent carried the dominant gene for white, one would have expected a 1 : 1 ratio. The excess of yellow plants may be due to an unusually high amount of

TABLE 4. *Russell-lupin. Segregation of white—yellow in acyanic plants.*

	Number of families	Number of plants	
		white	yellow
1. yellow × yellow	5		149
» selfed	5		87
2. white × white	1	21	
» × »	1	18	3
» selfed	2	24	
» »	3	18	13
3. yellow × white	9	54	93
white × yellow	3	60	55
» × »	1	21	
4. red × yellow	3		49
yellow × red	7		172
» × »	4	21	133
5. yellow × purple	6		111
white × purple	2	10	
6. red × white	3	7	14
white × red	5	43	33

selfing in these crosses, but it may also be due to an incomplete dominance of white, allowing the development of some yellow colour in the heterozygotes. This problem is mentioned further below.

As in the *L. polyphyllus* crosses, plants with cyanic flowers had cyanic stems, while those with acyanic flowers had acyanic stems.

d. Inheritance in the cross L. polyphyllus × Russell-lupin

Blue *L. polyphyllus* plants crossed to reddish and yellow plants of *Russell-lupin* and white *L. polyphyllus* crossed to reddish *Russell-lupin* gave in every case an F_1 with bluish flower colour (Table 5). In the crosses involving white or yellow, F_2 showed segregation of 3 cyanic : 1 acyanic in agreement with the results obtained in the crosses within the two species. There is a 3 : 1 segregation of bluish vs. reddish flowers indicating that the orange, red and pink types all lack the gene *B* which was assumed in *L. polyphyllus* to differentiate blue from pink. Crosses between the different reddish colour types have never given bluish colour. The genetics of the differentiation in orange, red and pink in the reddish group has not been studied.

The acyanic group contains a whole range of tones, from intense yellow to pure white, the yellow colour often being so light that it can scarcely be distinguished from white, and the separation of yellow from white has been very difficult. Yet it is obvious that different crosses have given different results. F_2 of the families 48, 52 and 53 gave in F_2 213 white : 91 yellow, which may be a 3 : 1 ratio in agreement with the results obtained in the *Russell-lupin*. Intercrosses of yellow F_1 plants in family 35, gave, however, a ratio of 1 white : 1 yellow. These deviating results may be due to an incomplete suppression of yellow in the heterozygotes. The manifestation of the yellow in the flowers may also have been influenced by modifiers segregating in these crosses.

F_1 plants from the crosses within *L. polyphyllus* were crossed to yellow and reddish *Russell-lupin* and some of the offsprings were tested in the next generation, (Table 6). The segregations cyanic : acyanic and bluish : reddish are in agreement with earlier results. In the acyanic group selfings of white plants have given 21 white : 77 yellow and selfings of blue and reddish only a small percentage of white plants. The possibility of the presence of a recessive white type cannot be excluded in this material. Probably, the varying results are due to a variable degree of suppression of yellow in the heterozygotes.

TABLE 5. *L. polyphyllus*×*Russell-lupin.*

Fam.	Parents	F_1	F_2(F_1 plants selfed and intercrossed)					
			cyanic			acyanic		
			bluish	reddish	total	white	yellow	total
31	*Russell*×*L. pol.* orange blue	bluish	216	78	294			
55	*L. pol.*×*Russell* blue red	»	19	5	24			
33	*Russell*×*L. pol.* orange white	»	8	3	11	1	3	4
48	*Russell*×*L. pol.* pink white	»	132	27	159	38	15	53
53	*L. pol.*×*Russell* white pink	»	28	6	34	6	2	8
246	*L. pol.*×*Russell* blue yellow	»	18	5	23	3	3	6
52	*L. pol.*×*Russell* white yellow	white				169	74	243
35	*L. pol.*×*Russell* white yellow	9 white 11 yellow						
	white F_1 plants from fam. 35 intercrossed					68	31	99
	yellow F_1 plants from fam. 35 intercrossed					194	195	389
	white and yellow F_1 plants crossed					99	98	197

Fam. 33, 48, 53, 246:
Obtained: 227 cyanic: 71 acyanic
Exp.: 223,5 » 74,5 » , $\chi^2 = 0{,}32$, $P = 0{,}5-0{,}7$.
Fam. 31, 55, 33, 48, 53, 246:
Obtained: 421 bluish: 124 reddish
Exp.: 408,75 » : 136,25 » , $\chi^2 = 1{,}47$, $P = 0{,}2-0{,}3$.

e. Inheritance in the cross L. nootkatensis×L. arboreus

Blue *L. nootkatenesis* was crossed with yellow *L. arboreus*. While in *L. nootkatensis* blue colour is present in stems and flowers, *L. arboreus* has, unlike any of those described earlier, the combination of cyanic stems and yellow flowers. However, a weak tinge of purple may occur in old flowers on some plants.

TABLE 6. *L. polyphyllus* × *Russell-lupin.*

Fam.	Parents	F_1	F_2 (F_1 plants selfed and intercrossed)					
			cyanic			acyanic		
			bluish	reddish	total	white	yellow	total
248	*L. pol.* × *Russell*	(1) 3 bluish						
	blue yellow	AaBb	15	3	18	1	5	6
	AaBb aabb	(2) 4 reddish						
		Aabb		31	31		11	11
		(3) 6 white						
		aabb				21	77	98
						22	93	115
252	L. pol. × *Russell*	23 bluish						
	blue pink	(1) AABb	96	46	142			
	AaBb AAbb	(2) AaBb	82	18	100	1	20	21
		20 reddish						
		(3) AAbb		92	92			
		(4) Aabb		145	145	2	49	51
						3	69	72

Fam. 248 (1), (2): Obtained: 49 cyanic: 17 acyanic
Exp.: 49,5 » 16,5 » $\chi^2 = 0{,}02$, $P = 0{,}8-0{,}9$
Fam. 248 (1): Obtained: 15 bluish: 3 reddish
Exp.: 13,5 » 4,5 » $\chi^2 = 0{,}67$, $P = 0{,}3-0{,}5$
Fam. 252 (2), (4): Obtained: 245 cyanic: 72 acyanic
Exp.: 237,75 » 79,25 » $\chi^2 = 0{,}88$, $P = 0{,}3-0{,}5$
Fam. 252 (1), (2): Obtained: 178 bluish: 64 reddish
Exp.: 181,5 » 60,5 » $\chi^2 = 0{,}27$, $P = 0{,}5-0{,}7$

The F_1 plants had all cyanic stems. The flowers were light bluish, slightly yellow near the base of the petals. In the F_2 generation, which was raised in one family, anthocyanin was present in the stems of all plants.

When the flowers are young, segregation for bluish : white and yellow may be distinguished. The numbers obtained are recorded in Table 7. This may be a 3 : 1 segregation, though there is a deficit of yellow and white flowered plants. It is possible that more plants of this type would have appeared among 275 plants which did not flower, even in their second year. Nearly all of the flowers which were white or yellow when young, developed more or less of a bluish colour as they grew older. The colour of most bluish flowers was light, though the whole range could be found, from very light ones to almost the same intensity as in

L. nootkatensis. The colour was often unevenly distributed in the petals, the blue colour being frequently mixed with yellow.

f. Inheritance in the cross L. polyphyllus×L. arboreus

Blue and pink *L. polyphyllus* were crossed with yellow *L. arboreus*. With respect to colour of stems and flowers, this is a combination of the same types as in the preceding cross.

The F_1 plants had all cyanic stems and light bluish flowers. The F_1 plants were intercrossed and crossed with red and with yellow *Russell-lupin* (Table 7). In F_2 a segregation in the young flowers of 3 cyanic : 1 yellow or white was found, as in the preceding cross. A few of the yellow and white flowers showed a slight tinge of bluish colour as they grew old, though not as much as in the cross *L. nootkatensis*×*L. arboreus*. The cyanic flowers consisted of blue, purple and pink. Some flowers were very light with darker veins, and often the bluish colour was mixed with yellow.

Apparently *L. arboreus* carries a recessive gene reducing anthocyanin in the flowers, particularly at early stages. There appeared, however, 5 plants lacking anthocyanin both in flowers and stems, and 8 plants with coloured flowers but with no discernible anthocyanin in the stems. The colour of anthocyanin in the stems was often weak, and it is possible in this case that absence of anthocyanin was due to environmental influences. This can only be verified by testing the genotypes. The same types were also observed in a progeny obtained by crossing the F_1 plants to *Russell-lupin*.

g. Inheritance in the cross L. nootkatensis×Russell-lupin

Blue *L. nootkatensis* was crossed with yellow *Russell-lupin*. The F_1 plants had all cyanic stems and bluish flowers. In the F_2 generation, comprising only one family, a 3:1 ratio was obtained for cyanic:acyanic flowers (Table 7). The cyanic plants consisted of 68 bluish and 21 reddish ones, which is also in agreement with a 3 : 1 ratio. In this cross, the acyanic flowers were pure white or pure yellow, and the colour of cyanic flowers was not diluted as in the two preceding crosses.

The segregation found in this cross is as would be expected if *L. nootkatensis* carries the genes *A* and *B*, corresponding to what is found in *L. polyphyllus* and *Russell-lupin*. However, as in the preceding cross, plants with acyanic flowers and cyanic stems and with cyanic flowers and acyanic stems occurred. Also in this cross the possibility of incorrect classification, due to modifications of colour in the stems, may

TABLE 7. *Crosses including L. nootkatensis, L. arboreus, L. polyphyllus and Russell-lupin.*

Parents			F_1		F_2			
	flowers	stem	flowers	stem	stem	flowers		
						cyanic	acyanic	total
L. noot. × *L. arb.*	cyanic acyanic	cyanic cyanic	cyanic	cyanic	cyanic acyanic	519 0	134 [1] 0	653 0
					total	519	134	653
L. pol. × *L. arb.*	cyanic acyanic	cyanic cyanic	cyanic	cyanic	cyanic acyanic	65 8	20 [2] 5	85 13
					total	73	25	98
(F_1 L. pol. × L. arb.) × *Russell*	cyanic cyanic	cyanic cyanic			cyanic acyanic	20 0	3 2	23 2
					total	20	5	25
(F_1 *L. pol.* × *L. arb.*) × *Russell*	cyanic acyanic	cyanic acyanic			cyanic acyanic	7 1	5 3	12 4
					total	8	8	16
L. noot. × *Russell*	cyanic acyanic	cyanic acyanic	cyanic	cyanic	cyanic acyanic	69 20	7 24	76 44
					total	89	31	120

[1] Anthocyanin develops in old flowers.
[2] » may develop in some old flowers.

be considered. The difference between the results of the last three crosses and those of the crosses within and between *L. polyphyllus* and *Russell-lupin* indicated, however, that in the former there are genes affecting only flowers or only stems.

5. Pigments

Paper-chromatographic tests of anthocyanins were undertaken in the following material:

Russell-lupin, 3 red and 3 purple plants.

F_2 *L. polyphyllus*×*Russell-lupin*, 1 red and 1 purple plant. Crude extracts of the pigments were run in butanol-acetic acid water (30 : 5 : 10). Extracts of the red flowers gave 2 spots, a and b, on the chromatogram:

a: R_F value 0,30; b : R_F value 0,16.

Extracts of the purple flowers also gave 2 spots, c and d.

c: R_F value 0,16; d : R_F value 0,08.

To test the anthocyanidins the components were separated, hydrolyzed and run in acetic acid—hydrochloric acid—water (30 : 3 : 10), where the following R_F values were obtained:

a: 0,64; b: 0,46; c: 0,46; d: 0,28.

When run in m-cresol—acetic acid—hydrochloric acid (1 : 1 : 1), the following R_F values were obtained:

a: 0,92; b: 0,76; c: 0,76; d: 0,59.

As control purified pelargonin, cyanin and delphin were used and the R_F values and colours of these spots agree with those found for spot a, spots b and c, and spot d respectively. The red and purple flowers both seem to contain a derivate of cyanidin, and in addition the red ones contain a derivate of pelargonidin, while the purple ones contain a derivate of delphinidin. The three pigments may be the corresponding glycosides of the anthocyanidins mentioned, namely cyanin, pelargonin and delphin. This suggests that the function of the gene *B* is to control the oxidation of the anthocyanin.

Tests for flavones were undertaken in the following material:

L. polyphyllus, 6 blue, 5 pink and 4 white plants
» *arboreus*, 2 plants
Russell-lupin, 2 purple, 2 red and 4 yellow plants
F_2 *L. polyphyllus*×*L. arboreus*, 6 plants
» » » ×*Russell-lupin*, 6 plants.

By running the crude extracts in butanol—acetic acid—water (30 : 5 : 10) one pigment showed a spot on the chromatogram with an R_F value of about 0,10, which appeared yellow over ammonia vapour in ultra-violet light. After hydrolyzation, by running in the same solvent it gave a greenish yellow spot with an R_F value of 0,86.

This pigment was present in all *L. polyphyllus*, in all *L. polyphyllus* × *L. arboreus*, and in 4 of the *L. polyphyllus* × *Russell-lupin* hybrids, but neither in *L. arboreus* nor in *Russell-lupin*. Except for this pigment, the flavones detected seemed quite uniform, irrespective of species and flower colour, and therefore the production of yellow colour could not be attributed to any particular pigment.

Extracts of the petals with petroleum ether indicate that yellow plastids exist in *L. arboreus*, and in yellow, orange and purple *Russell-lupin*, while this could not be detected in pink and blue flowers. It is likely that plastids are responsible for the greater part of the yellow colour in *L. arboreus* and *Russell-lupin*, and also for a part of the variation in the reddish and bluish types of *Russell-lupin*. But it is also possible that flavones as well are working in some of the colour types.

III. DISCUSSION

It was thought that the crosses might throw some light on the supposed origin of *Russell-lupin* from the cross *L. polyphyllus* × *L. arboreus*. The results of the crosses *L. polyphyllus* × *L. arboreus* described here have given no proof of this theory, but on the other hand, they do not contradict it. The conclusion from the cytological investigation is that the chromosomes of *L. polyphyllus*, *L. arboreus* and *Russell-lupin* are fairly homologous. The fertility in the hybrids *L. polyphyllus* × *L. arboreus* and in the backcross to *Russell-lupin* also agrees with the theory.

The growth habit of the *Russell-lupin*, which resembles that of *L. polyphyllus* but with a more compact growth and more branched stems, has been found among the F_2 plants of the cross *L. polyphyllus* × *L. arboreus*.

A pigment, identified by ultra-violet light on the paper chromatogram, but with no apparent effect on the flower colour, is found in *L. polyphyllus*, but neither in *L. arboreus* nor in the *Russell-lupins*. The six F_2 plants of the cross *L. polyphyllus* × *L. arboreus* tested all contained this pigment, but the number is too small to draw any conclusion, and one can expect to find plants free of this pigment in a larger F_2 family.

The genetic data for the flower colour are also too meagre, as they are based on only two families with a small number in the F_2 generation. Different tones of blue and pink exist also in *L. polyphyllus*, and other parent plants might have introduced other tones into hybrids. Representatives of the main groups of colour in the *Russell-lupin*, bluish, reddish, white and yellow, are to be found in the F_2 generation, but the colours are not so pure and dark as in the *Russell-lupin*. There is a great variability in flower colour in both crosses involving *L. arboreus*. Types with as large flowers and as compact spikes as in the *Russell-lupin* did not appear, and should not be expected as an immediate result from these crosses, as both parents had looser spikes and smaller flowers than *Russell-lupin*. However, these types may have been introduced from other types of the same species, as also with regard to these characters, there is variation within the species. The *Russell-lupin* is probably the result of cross breeding and selection for a long period and it is not impossible that it might have arisen by selection in a hybrid material from the cross *L. polyphyllus*×*L. arboreus*. The possibility of new mutations may also be considered.

SUMMARY

(1) Hybrids were obtained in the following crosses:

L. polyphyllus ×*L. arboreus*
» » ×*Russell-lupin*
» *nootkatensis*×*L. arboreus*
» » ×*Russell-lupin*

The cross *L. polyphyllus*×*L. luteus* was unsuccessful.

(2) All hybrids obtained were fully fertile, and their meiosis quite regular, with 24 bivalents. During meiosis the chromosomes often lay in pairs or in groups of 3 or more.

(3) In *L. polyphyllus, Russell-lupin* and their hybrids there is a single gene *A* for cyanic flowers. In the F_2 generation of the cross *L. nootkatensis*×*Russell-lupin* a 3: 1 ratio for cyanic: acyanic flowers was also obtained. The crosses *L. nootkatensis*×*L. arboreus* and *L. polyphyllus*× *L. arboreus* indicate a monohybrid segregation for acyanic—cyanic flowers at an early stage. Nearly all acyanic flowers of the former and a few of the latter cross developed bluish colour in various degrees at later stages. Some colour was also observed in old flowers of *L. arboreus*, but not as much as in the F_2 segregates. In the last three crosses antho-

cyanin in flowers and stems seem to have segregated independently of each other, which is not the case in *L. polyphyllus* and *Russell-lupin.*

(4) In *L. polyphyllus* and in the cross *L. polyphyllus*×*Russell-lupin* the blue colour of cyanic flowers is dependent on one gene *B*, giving bluish and *b* reddish flower colour. A 3 : 1 ratio for bluish—reddish colour is also obtained in F_2 of the cross *L. nootkatensis*×*Russell-lupin.*

(5) The crosses in *Russell-lupin* indicate a single gene segregation for white—yellow flower colour, white being dominant to yellow. In the crosses *L. polyphyllus*×*Russell-lupin*, however, selfing and intercrosses of yellow plants have segregated plants with white flowers. The existence of a recessive white type cannot be excluded, but it is most probable that the varying results are due to a variable suppression of yellow colour in the heterozygotes.

(6) Paper-chromatographic tests indicate that *AB* plants of *Russell-lupin* contain cyanidin- and delphinidin-derivatives, while *Ab* plants contain cyanidin- and pelargonidin-derivatives.

The yellow colour seems at least partly to be due to plastids possibly also to flavones.

Acknowledgements. — I am indebted to Professor Dr. H. WEXELSEN and to members of the staff at John Innes Horticultural Institution, especially to Dr. D. LEWIS for help and advice. Part of the work was made possible by a scholarship from the Agricultural Research Council of Norway, for which I wish to express my thanks.

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THE SEX CHROMOSOMES IN *HUMULUS*

By *PETER JACOBSEN*
BOTANICAL INSTITUTE, UNIVERSITY OF COPENHAGEN

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I. INTRODUCTION

THE sex chromosomes of the two *Humulus* species have been the subject of studies by several scientists since they were first detected by WINGE (1923). Even though their existence has been accepted or re-detected, the interpretation as to which is X and which is Y, and of homology conditions have given rise to some discussion. As long as a reliable interpretation of the sex chromosomes at mitosis was lacking, a continuation of the work with regard to the type of sex determination was impracticable.

The purpose of the present work is to explain — by means of a modernized technique — the situation of the sex chromosomes at meiosis and to render possible a reliable identification at mitosis with a view to attempting subsequently to clarify the sex determination type by means of chromosome doubling, crossing and cytological investigations.

The plants of *Humulus japonicus* that were used came from seed bought at the firm of J. E. Ohlsens Enke, Copenhagen, and produced in France. The plants of this species, which is strictly dioecious, are grown in flowerpots in hothouses after an attempt to grow them outdoors had failed, the plants coming into flower so late in the autumn that seed did not develop.

Of *Humulus lupulus*, plants were used raised from cuttings of plants grown in the botanic garden of the University of Copenhagen and from escaped plants outside the garden. *H. lup.* is grown outdoors, but cuttings are rooted in flower-pots for root tip mitoses.

Several cytological methods have been tried. The method preferred for investigation of meiosis is fixation in Carnoy 9:6:1 and aceto orcein squash. The investigation of mitosis has given the best results by using the following method: Root tips are pre-treated in 0.001 mol 8-hydroxychinoline at 10—12° C for 3—4 hours. Fixation in 30 %

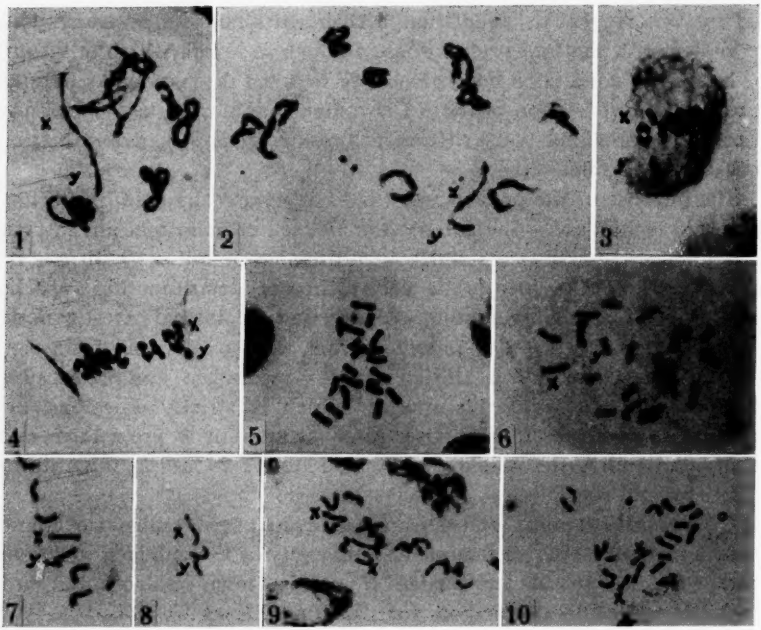
alcohol with 1 % lactic acid at — 5° C for at least 12 hours. Stain with fuchsine according to Feulgen. Squash in 10 % acetic acid and mount in Canada balsam.

II. CYTOLOGICAL OBSERVATIONS

1. Humulus lupulus

a. Meiosis

Meiosis in the male plant can be followed in all phases, and the sex chromosomes are identifiable at diplotene, diakinesis, metaphase I and anaphase I (Figs. 1—4). Pachytene was studied very closely, but it was not possible to identify the sex chromosomes at this stage. Diplotene, on the other hand, supplies a very clear picture of X and Y, and structural differentiation is seen along the two chromosomes, which always



Figs. 1—10. *Humulus lupulus*. — Figs. 1—4. Meiosis in the male plant. — Fig. 1. Diplotene showing X and Y in end-to-end conjugation (see also Fig. 11). — Fig. 2. Diakinesis. — Figs. 3—4. Metaphase I. — Figs. 5—8. Mitosis in the male plant. — Fig. 5. Metaphase. — Figs. 6—8. Prometaphase. — Figs. 9—10. Mitosis in the female plant, prometaphase. — The mitosis chromosomes were pre-treated with 8-hydroxychinoline. — ×1000.

appear conjugated end-to-end (Fig. 1). A distinct differentiation in length between the two chromosomes is apparent, which can be observed at all stages. Starting at diakinesis, the visible structural differences gradually disappear, and only the difference in length in connection with the end-to-end conjugation makes it possible to follow the X—Y pair (Figs. 2—4). To determine which of the chromosomes is X and which is Y is, however, not feasible, since it has not been possible to identify the sex chromosomes at meiosis in the female plant and to determine whether they are homologous with the long or the short chromosome in the male plant. Studies of the mitosis succeeded in providing the clarification required.

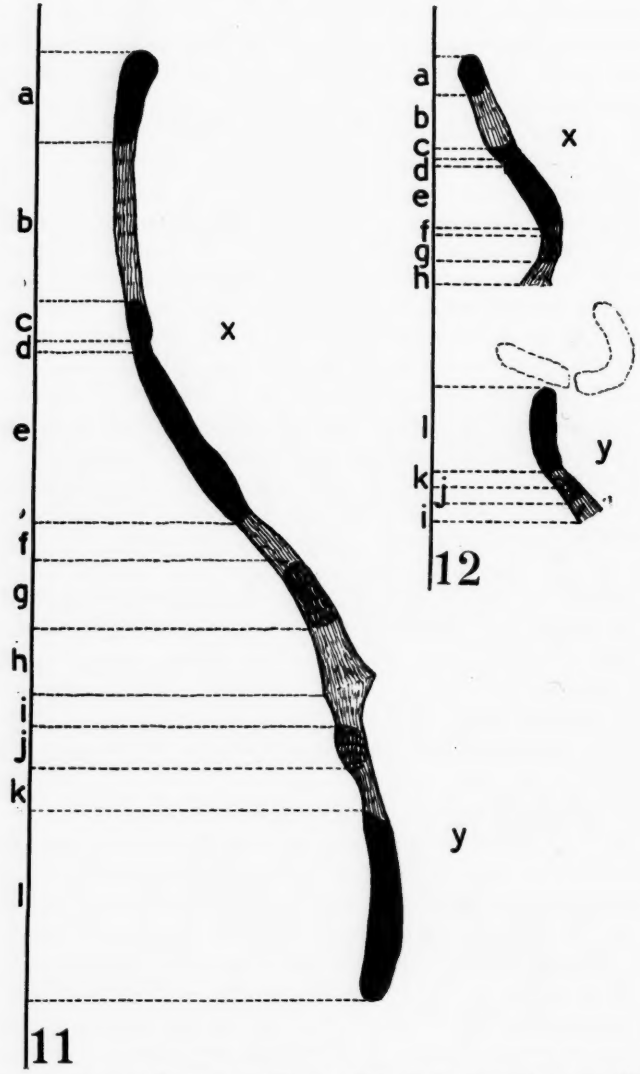
b. Mitosis

The metaphase of the mitosis shows 20 chromosomes varying somewhat with respect to length (Fig. 5). As previously attempted in other works, a measurement was made of the length of the chromosomes in good micro-photographs, but without any clear result. Investigations of the prophase and the prometaphase of the mitosis by 8-hydroxychinoline (TJIO and LEVAN, 1950), showed that the sex chromosomes can be identified at these phases by means of various constant structural characteristics. In the male plant one medium-sized chromosome is found in which the middle-section of the short arm, after staining according to Feulgen, is considerably less stainable than the rest of the chromosome (Figs. 6—8). In the female plant are found 2 chromosomes that are structurally homologous with this chromosome (Figs. 9—10). Consequently, this chromosome must be interpreted as the X chromsome.

After this observation a closer study of sizes and variations in length of the arms of the chromosomes of the male plant made it possible to establish that the male plant has 3 chromosomes, which are shorter than the other chromosomes in the complement. One of these chromosomes differs from the 2 others by its short arm being shorter and ending diffusively (Figs. 6—8). This chromosome is not represented in the female plant, and it must consequently be interpreted as the Y chromosome. Thus the Y chromosome is the smaller chromosome of the two heteromorphic sex chromosomes observed at meiosis in the male plant.

c. Homologous and differential segments

With the result of the studies of the mitosis as a basis and by reverting to meiosis, interpretations can be made with regard to homologous



Figs. 11—12. *Humulus lupulus.* — Fig. 11. Meiosis in the male plant. Diplotene showing end-to-end conjugation of X and Y and the difference in structure along the chromosomes (see text). — Fig. 12. Mitosis in the male plant. The circumscribed sections a, b, c, etc. correspond as regards homology to sections in Fig. 11 referred to by the same letters. Both figures semi-schematical after Figs. 1. and 8. — ×6000.

and differential segments in the sex chromosomes. By looking at Figs. 1 and 8 and the corresponding semi-schematic drawings, Figs. 11 and 12, it will be possible by means of the segments *a*, *b*, *c*, etc., drawn in the figures, to find the corresponding segments of the meiosis at mitosis, and vice versa. The kinetochore of the X chromosome is in *d*, and that of the Y chromosome is in *k*. The position of the kinetochore at meiosis for the two sex chromosomes has been calculated on the basis of its position in the mitotic chromosomes, but the consequent complete agreement between the sequence of the various structural characteristics of the sex chromosomes at meiosis and mitosis makes it justifiable to consider as correct the orientation of X and Y at meiosis that has been given. From this it follows that the homologous segments must comprise segment *h* in the X chromosome and segment *i* in the Y chromosome; whether segment *g* in X and segment *j* in Y are wholly or partly included in the homologous segment cannot be determined with certainty, for segment *k* in the Y chromosome contains the kinetochore and, consequently, cannot be homologous with segment *f* in the X chromosome. As, at the same time, only end-to-end conjugation of the sex chromosomes has been observed, even at earliest diplotene, it must be assumed that, as far as the Y chromosome is concerned, the homologous segments comprise, at most, the short arm, and as far as the X chromosome is concerned, at most, the segments of *h* and *g*. Thus the homologous segments will comprise, in the case of the Y chromosome, not more than one fourth, and in the case of the X chromosome, not more than one sixth of the entire length of the chromosome.

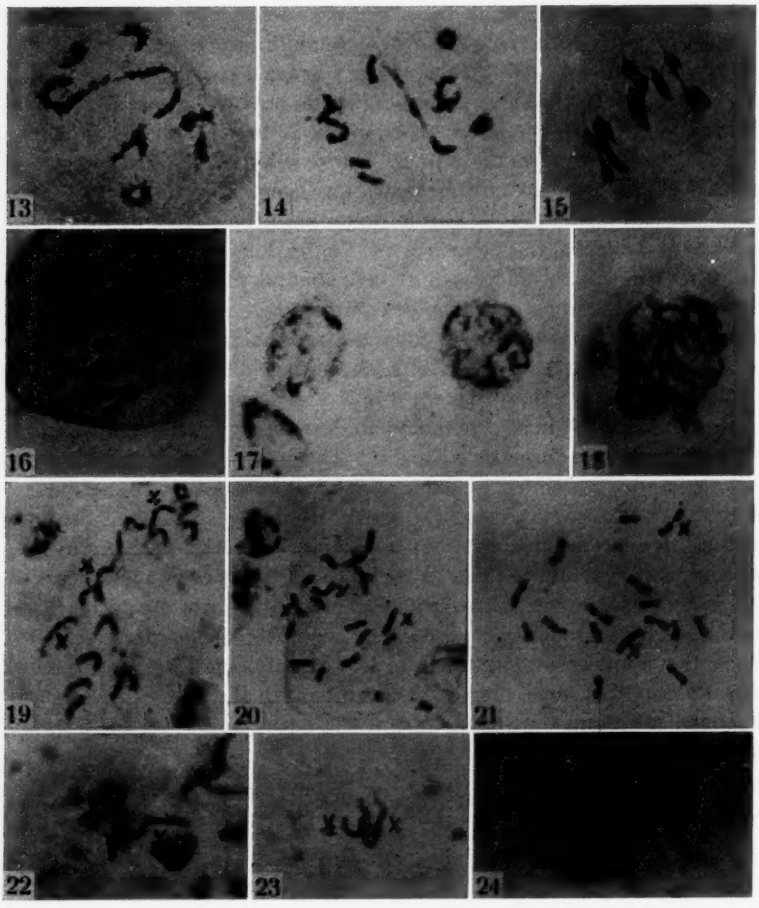
2. Humulus japonicus

a. Meiosis

At meiosis in the male plant a trivalent chromosome figure is clearly seen, which can be observed at late diplotene, at diakinesis, metaphase I, and anaphase I (Figs. 13—16). At anaphase I it is seen that the arrangement is 8 chromosomes to one pole and 9 chromosomes to the other. However, studies of the meiosis do not provide possibilities of identifying with certainty the individual sex chromosomes in *H. japonicus* either. Also in this case this may be done by studies of the mitosis.

b. Mitosis

The metaphase of the mitosis in the male plant shows 17 chromosomes and in the female plant 16 chromosomes. (Figs. 20 and 21). In



Figs. 13—24. *Humulus japonicus.* — Figs. 13—16. Meiosis in the male plant. — Fig. 13—14. Diakinesis showing the heterochromatic Y chromosomes conjugated end-to-end with either end of X. — Figs. 15—16. Metaphase I. In the trivalent figure of sex chromosomes X proceeds to one pole and the two Y chromosomes to the other. — Figs. 17—20. Mitosis in the male plant, showing the Y chromosomes in resting nuclei (Fig. 17), at early prophase (Fig. 18) and prometaphase, where X, too, is identifiable (Figs. 19—20). — Fig. 21. Mitosis-metaphase in the female plant. — Figs. 22—24. Mitosis in the male plant. — Fig. 22. The Y_1 and Y_2 chromosome, showing the structural difference of the four end segments (see also Fig. 27). — Fig. 23. Y_2 and X with the homologous end segments facing upwards on both (see also Fig. 25). — Fig. 24. Y_1 and X. The homologous end segments will be found by comparison with Fig. 26. — The mitosis chromosomes were pre-treated with 8-hydroxychinoline. — ×1000.

H. jap. it can be established by measurements of the chromosomes on good metaphase plates that the male plant has three chromosomes distinctly longer than the rest of the chromosomes in the complement (Fig. 20). The female plant has only two chromosomes distinct from the others in size (Fig. 21). The kinetochore of the two large chromosomes of the female plant is of submedian location. In the male plant the kinetochore of one of the long chromosomes is of median location whereas in the two others it is of submedian location, but any safe indication is not provided by these characteristics. Also here studies of prophases and prometaphase after pre-treatment with 8-hydroxychinoline proved to provide the desired clarification.

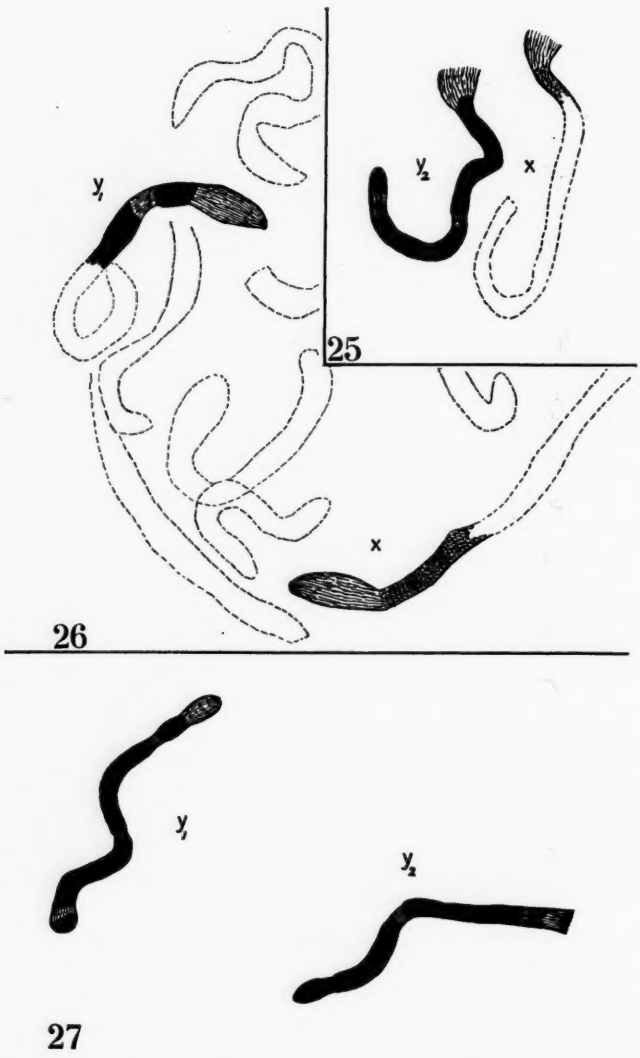
The resting nucleus of the male plant has two heterochromatic parts that are absent in the female plant, (Fig. 17). By following the cell division from its earliest stages it is found that these heterochromatic parts gradually reveal themselves as chromosomes, which in the prophase and up to and including the prometaphase prove to be clearly heterochromatic for the greater part of their length (Figs. 18—19). At metaphase and anaphase the stainability of these chromosomes is not greater than in the case of the remaining chromosomes of the complement. These chromosomes have been interpreted as Y chromosomes.

In good plates with fine fixation and levelling out of the cells it is found that the two heterochromatic Y chromosomes are visibly different in structure (Fig. 22). One, (Y_1) has a kinetochore of submedian location. The other (Y_2) has a kinetochore of median location. Each of the four chromosome ends has its own characteristic appearances, which is constant from cell to cell and from plate to plate, in so far as other circumstances, levelling out, identical phase and the unaffectedness of the chromosomes to the heavy levelling-out process are the same.

c. Homologous and differential segments

Here as in the case of *H. lup.* an account of the homology relations between the two Y chromosomes and the X chromosomes has been attempted. In the case of *H. jap.* it is however very difficult, seeing that the X chromosome in the prophase is not only less stainable for the greater part of its length than Y_1 and Y_2, but also less stainable than most autosomes.

A probable explanation is the following: The female plant has no heterochromatic chromosomes. At diakinesis in the male plant it is seen that the two extreme chromosomes are stainable to a greater



Figs. 25—27. *Humulus japonicus*. — Fig. 25. Y_2 and X. Semi-schematically after Fig. 23 is shown the position of the homologous segments; the same has been done in the case of Y_1 and X in Fig. 26 (semi-schematical after Fig. 24). — Fig. 27. Y_1 and Y_2. After Fig. 22 the difference of structure and morphology are shown semi-schematically. — ×6000.

degree than the middlemost one and pair with the latter end-to-end (Figs. 13 and 14). The sequence must therefore be Y_1XY_2. Consequently, it is to be expected that a shorter or longer end segment of Y_1 and Y_2, respectively, is homologous each with its corresponding end segment of X. Y_2 has only one non-heterochromatic end segment on one arm and, as at the same time a resemblance in structure between this segment and an end segment of the long arm of X can be ascertained, it follows that these segments must be homologous (Figs. 22+23 and 25+27). In Y_1 both end segments are non-heterochromatic, but here a resemblance of structure between the end segment of the long arm of Y_1 and the short arm of X indicates that the homologous segments are to be found here (Figs. 22+24 and 26+27).

To re-discover and to identify the individual chromosome segments from the chromosomes of the mitosis at diplotene or diakinesis, as was done in the case of *H. lup.*, has not been possible here. It is, however, likely that the homology does not extend beyond the non-heterochromatic end segments of Y_1 and Y_2 that pair with X. (The possibility should, however, not be left out of consideration that a segment homologous with a heterochromatic segment either in Y_1 or Y_2 but located in the X chromosome may present a different stainability here). The homologous segments of Y_1X and Y_2X, respectively, will thus amount to less than one sixth of the extent of each individual chromosome.

III. DISCUSSION

1. Technique

In a work of this type the cytological technique sets the limit to what can be attained as regards observations and the reliability of such observations. Great progress has been made in the field of technique since WINGE first attempted to clarify the situation of the sex chromosomes in *H. lup.* in 1923. In the present work, especially TJIO and LEVAN's method of pre-treating somatic cells with 8-hydroxychinoline has been of extreme importance. It makes it possible to get many nuclei into exactly the phase of division required, while at the same time the structure is clarified and the chromosomes are shortened. The shortened chromosomes will spread more easily so as to be clear of each other. On the whole, the squash technique with Feulgen staining makes it possible to observe structures in the chromosomes which will not be otherwise seen. Especially the difference of structure and stainability of the prophase chromosomes is very useful for the identi-

fication of certain chromosomes. However, it should be borne in mind that precisely in this phase chromosomes are very easily deformed through mechanical influence.

2. Previous works

WINGE showed the presence of heteromorphic sex chromosomes in the two *Humulus* species in 1923, but found at that time that both species had a simple X—Y mechanism. In 1928, however, KIHARA showed that an Y_1XY_2 mechanism was found in *H. jap.* and thought that it must be identical with the same mechanism detected in *Rumex* in 1923. WINGE took up the question once more and agreed with KIHARA that there was no simple X—Y mechanism in *H. jap.*, but he refused, however, to recognize KIHARA's interpretation. WINGE found that in the first metaphase of the reduction division the 3 sex chromosomes were as often found seriated as they were found in V-shape arrangement, in which case the central chromosome, X, proceeds to one pole, the two extreme ones, Y_1 and Y_2, proceeding to the other pole. WINGE objects that if the three chromosomes are not homologous, the sequence of XY_1Y_2 will be required to get the proper distribution of X, Y_1 and Y_2 in the cases of a seriated arrangement of the chromosomes. X is then to proceed to one pole and Y_1Y_2 to the other pole. Otherwise a balanced mechanism of sex distribution will not be maintained, and pollen will appear with XY_1 and XY_2 besides X and Y_1Y_2.

KIHARA's observations showed that the V-shaped figure was far the most frequent one, and the X—Y_1Y_2 distribution was a natural interpretation to him. WINGE's observations showed that the V-shaped and the seriated figure were almost equally frequent and would therefore only find an X—XX distribution natural, seeing that the V as well as the seriated figure would give this distribution. This 3X assumption leads WINGE to the inference that the larger sex chromosome in *H. lup.* must be the Y chromosome, it being his opinion that a »fusion» must have taken place here between the two X chromosomes in *H. jap.*, which thus come to form a genuine Y chromosome. WINGE's 3X assumption has been criticized by DARLINGTON and WESTERGAARD and is now only of historical interest.

In KIHARA and HIRAYOSHI's final work on sex chromosomes in *H. jap.*, 1932, these have been identified somatically as the three longest chromosomes in the complement. The kinetochore in the X chromosome is found to have a median position, and in Y_1 and Y_2 the position is submedian. The pachytene and the diplotene have been investigated

and figures have been found which have been interpreted as the conjugated sex chromosomes. In a schematical drawing it has been shown how the authors visualize the homology situation of the three sex chromosomes. The arms of the X chromosome, which are of equal length, are homologous with the long arm of Y_1 and Y_2. As there is no particular affinity between the arms of the two Y chromosomes which do not pair with X, they must be differential as regards X and not homologous. It should be observed, however, that the homology between the X and the 2 Y probably only comprises a larger or smaller section of the distal end segments.

SKOVSTED attempted (1953) to identify the sex chromosomes in the two *Humulus* species somatically. In *H. jap.* somatic chromosomes have been registered in the female as well as in the male plant. The chromosomes of the male plant have been arranged in a diagram, and SKOVSTED finds that the chromosomes that have been repeated three times are the three smallest ones. SKOVSTED does not mention that this is at variance with WINGE's results as well as those of the Japanese scientists.

DERENNE dealt with sex chromosomes in *H. lup.* in 1954. The heteromorphic sex chromosomes in the male plant were found at meiosis, but he thinks it impossible to show whether it is the X or Y which is the larger chromosome unless a plant can be produced with XXY or XXXY.

3. The present work

In the case of *H. jap.* the results presented in this work are on the whole in agreement with what KIHARA and HIRAYOSHI presented in their work of 1932. There is a disagreement as regards the position of the kinetochore and the three sex chromosomes. KIHARA and HIRAYOSHI state that it is the X chromosome that has a median kinetochore, whereas it has been found here that it is Y_2. Even if racial differences are not to be left out of consideration, there is probably more reason in assuming that KIHARA's technique was not fully as good as the one used here. Nor did he observe that the Y chromosomes were heterochromatic, which has been of almost indispensable assistance in avoiding confusion with the X chromosome.

Regarding the dispute between WINGE and KIHARA about the frequency of the appearance of the V-shaped and the seriated orientation of the three sex chromosomes it may be said that in the material used here the V-shaped orientation has been entirely prevalent, seeing that

the seriated orientation has only been observed with certainty three times in a large number of cells from many different plants. In addition, there were a number of cases in which the mode of orientation was difficult to decide. In the diploid plants of *H. jap.* examined (>200), only one plant was found to have a diverging sex chromosome complement, namely XXY_1. It is conceivable that it is the result of a reduction division in the male plant after seriation so that Y_1X has gone to one pole and Y_2 to the other.

SINOTO (1929) and ONO (1937) found a tetravalent sex chromosome arrangement in *H. lup.* from Japan. Further, by studying meiosis in various races of *H. lup.* with partly bivalent and partly tetravalent sex chromosomes, ONO found, 1955, that there was a certain likelihood that the larger chromosome in the race with bivalent sex chromosomes is the X chromosome. No opinion is to be given here regarding this problem since it is, to all appearances, a question of Japanese races deviating from the Danish race investigated here, which shows bivalent sex chromosomes in all cases. A preliminary investigation of plants from seed from Japan has shown that a chromosome exists which with regard to morphology corresponds exactly to the X chromosome found in the Danish race. In some plants it is represented twice, in others only once. A Y chromosome of an appearance similar to that found in the Danish race is apparently lacking. As the plants have not yet come into flower, a final investigation, which must also be based on meiosis, cannot be made until next year.

It has not been possible to find any morphological resemblance between the sex chromosomes in the two *Humulus* species, and it is doubtful whether it would be a justifiable theory that either of the two sex chromosome mechanisms in their present form could be derived from the other. The presence of heterochromatic, probably partly inert, Y chromosomes indicates a long independent development of this mechanism.

SUMMARY

(1) In *H. lupulus* and *H. japonicus* the sex chromosomes have been identified and described at meiosis as well as at mitosis.

(2) *H. lup.* has an XY mechanism, in which the X chromosomes is clearly longer than Y and of medium size as regards the autosomes.

(3) *H. jap.* has a Y_1XY_2 mechanism, in which the three sex chromosomes are clearly longer than the autosomes. The Y chromosomes are heterochromatic for the greater part of their extent.

(4) By studying prophase and prometaphase at mitosis various structural characteristics have been found, which have made it possible to describe in the case of both species the position and the extent of homologous and differential segments and, consequently, the orientation of the chromosomes during conjugation.

Acknowledgement. — This work was carried out partly at the Genetic Institute and partly at the Botanical Institute of the University of Copenhagen. It was rendered possible through financial support from the Danish State's Research Foundation obtained for that purpose by Professors KNUD JESSEN and TYGE W. BÖCHER, Botanical Institute of the University. For this and for the interest shown in my work by these gentlemen as well as by Professor M. WESTERGAARD, Head of the Genetic Institute, I wish to express my best thanks.

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EFFECTS OF NEUTRONS AND CHRONIC GAMMA RADIATION ON GROWTH AND FERTILITY IN OATS AND BARLEY

By *KNUT MIKAELSEN* and *KNUT AASTVEIT*
INSTITUTE OF GENETICS AND PLANT BREEDING, AGRICULTURAL COLLEGE OF NORWAY, VOLLEBEKK, NORWAY
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I. INTRODUCTION

IT is a well-established fact that ionizing radiation seriously interferes with growth and fertility of living organisms. In higher plants a number of reports (BJØRNSETH, GOKSØYR and MIKAELSEN, 1957, CALDECOTT, FROLIK and MORRIS 1952, EHRENBERG and NYBOM 1954, EHRENBERG and SÆLAND 1954, GRANHALL, EHRENBERG and BORENIUS 1953, MAC KEY 1952, 1954, MIKAELSEN 1956 among others) have demonstrated pronounced retardation of seedling growth and reduced seed-setting after exposures of ionizing radiations. Various kinds of ionizing radiations may not affect growth and fertility in the same manner. Differences in sensitivity to radiation may also depend on the chromosome complement. Thus, MÜNTZING (1941, 1942) found that diploid and tetraploid barley were differently affected by X-irradiation. Similar differences were also found between diploid, tetraploid and hexaploid forms of yeast (SAX 1955). MAC KEY (1952, 1954) found differences in sensitivity between the hexaploid wheat and the diploid barley species.

In the present investigations the effects of neutrons and chronic gamma radiation on growth and fertility in barley ($2n=14$) and oats ($2n=42$), are studied. The oat variety, *Sisu*, and the two-rowed barley variety *Maja* were used as experimental material. Seeds were irradiated in the centre of the heavy water reactor, *Jeep*, which is run by the *Joint Establishment of Nuclear Energy Research* at Kjeller, Norway. The seeds were placed in cylindrical aluminium tubes and enclosed in cylindrical graphite boxes. The walls of the graphite boxes were 5 mm thick. The reactor was operated at *10 KW*.

In the centre of the pile there are thermal, epithermal and fast neutrons of different energies and gamma radiation. Detailed descriptions

and definitions of the radiations in this positions are given elsewhere (EHRENBERG and SÆLAND 1954 a and b, MIKAELSEN, BJØRNSETH and HALVORSEN 1956). The main part of the radiation consists of thermal neutrons. From EHRENBERG and SÆLAND's (1954 b) calculations, however, the lesser dose of non-thermal neutrons is responsible for 70—80 per cent of the total radiation dose. The contaminating gamma radiation can be almost neglected since it will only give an unimportant contribution to the total radiation dose.

The radiation doses are determined by chemical dosimetrical methods (EHRENBERG and SÆLAND 1954 a and b). According to these measurements the barley received the following doses: 600, 1200 and 1800 reps. The oat seeds were exposed to 1200, 2400 and 3600 reps. After the neutron irradiations the seeds were planted in the field within a few days.

The chronic gamma radiation doses were given in a »*Gamma field*». In this field the plants were grown from germination to maturity and continuously exposed to gamma radiation from a 25 Curie Co^{60} isotope (the source was extended to 50 Curie in 1956). The radiation source is placed in the centre of the field. The installation and operation of the field is described elsewhere (MIKAELSEN 1956). The oats and barley plants were grown in concentric arcs around the source. At different distances from the source the plants received various doses of gamma radiation ranging from 5 r/day to 200 r/day.

The effects of neutron and chronic gamma radiation on growth were determined on plant height, number of straws and number of flowers per head. Plant fertility was determined as the percentage of fully developed kernels of the total number of flowers per head.

II. EXPERIMENTAL RESULTS

1. Effects of Neutrons

a. *Growth and development.* As also demonstrated in other experiments [BJØRNSETH, GOKSØYR and MIKAELSEN (1957), MIKAELSEN, BJØRNSETH and HALVORSEN 1956], germination proper was not affected by the neutron doses given. The growth rate, however, was definitely affected in both oats and barley. In barley, doses of 600 and 1200 reps did not affect the height of the mature plants, since no retarding effect as compared with untreated controls could be demonstrated (Table 1). After a dose of 1800 reps, however, the plants were very seriously af-

TABLE 1. *The effects of neutrons on growth and fertility in oats and barley.*

Doses of neutrons	No. of plants examined	Plant heights in cm	No. of straws per plant	No. of flowers per head	Percentage of seed setting
Oats:					
Control	20	117.2 ± 1.3	3.6	126.7 ± 3.0	95.2 ± 1.0
1200 reps	20	112.4 ± 2.0	2.2	94.7 ± 6.7	89.0 ± 2.8
2400 reps	20	107.8 ± 2.3	2.7	98.1 ± 8.7	54.7 ± 6.0
3600 reps	20	80.7 ± 3.1	3.6	49.2 ± 5.5	27.4 ± 8.8
Barley:					
Control	16	42.7 ± 2.3	2.8	14.5 ± 1.1	98.6 ± 1.0
600 reps	25	45.5 ± 2.4	3.3	16.1 ± 0.9	73.2 ± 6.6
1200 reps	27	43.8 ± 2.9	2.6	15.6 ± 0.7	35.2 ± 7.5
1800 reps	No survivors				

fected and no plants survived. After a few weeks the plants showed no growth and died. In oats a dose of 1200 reps seemed to exert a slight effect on plant height (Table 1). The reduction increased with doses of 2400 and 3600 reps.

Number of fully developed straws per plant seemed not to be impaired by neutron irradiation in oats and barley with the doses applied. (Table 1). In barley the number of flowers per head proved to be unaffected by neutron doses of 600 and 1200 reps. (Table 1). In oats the number of flowers per head was markedly and significantly reduced by doses of 1200—3600 reps. The effect increased with dosage and the reduction was approximately 60 % with a dose of 3600 reps (Table 1).

b *Effects on fertility.* Pronounced effects on seed-setting were clearly demonstrated (Table 1). A marked reduction in number of grains was obtained in both. It is obvious from Table 1 and Figure 1 that fertility was more seriously influenced by neutron irradiation in barley than in oats. The dose effect curve of barley shows a steeper fall than the concurrent curve for oats. In barley 1200 reps produced almost the same effect as 3600 reps in oats. In barley the fertility was reduced from 98.6 % in the controls to 35.2 % in the plants exposed to 1200 reps. In oats 3600 reps of neutron radiation reduced fertility to 27.4 % as compared to 95.2 % in the untreated control plants.

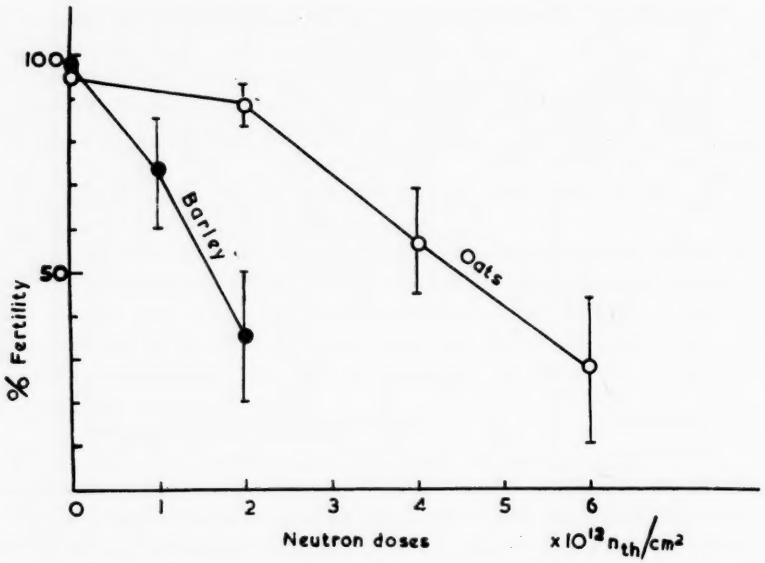

Fig. 1. Effects of various doses of neutrons on fertility in oats and barley.

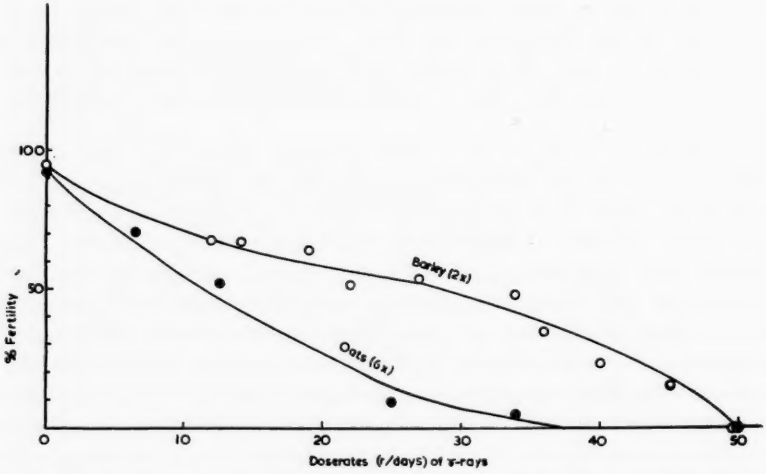

Fig. 2. Effects of various dose-rates of gamma radiation on fertility in oats and barley.

2. Effects of Chronic Gamma Radiation

a. *Effects on growth.* The barley plants irradiated with dose-rates from 6.25 r/day to 73 r/day showed no effect on plant height. From Table 2 it may seem as if the medium doses had stimulated plant growth, but no significance was paid to this finding. The same feature has appeared every year, but this may be due to the soil heterogeneity around the radiation source. Dose-rates exceeding 73 r/day were also applied, but no survivors were obtained.

In oats, which was exposed to dose-rates of 12.5 r/day to 100 r/day, a marked and significant reduction in plant height was obtained with

TABLE 2. *Effects of chronic gamma radiation during 108 days on growth and fertility in oats and barley.*

Doses of gamma radiation	No. of plants examined	Plant height in cm	No. of straws per plant	No. of flowers per head	Percentage of seed setting
Oats:					
0	20				91.5 ± 1.0
12.5 r/day	141	93.5 ± 1.1	2.6	28.9 ± 1.4	51.9 ± 2.5
25.0 „	35	101.1 ± 3.4	3.3	22.7 ± 1.7	9.3 ± 2.8
34.0 „	58	97.6 ± 2.7	3.9	29.2 ± 2.0	4.8 ± 1.2
50.0 „	44	86.7 ± 2.4	3.6	28.6 ± 2.2	0.1
68.0 „	18	71.3 ± 4.0	4.6	15.3 ± 2.0	0
100.0 „	20	54.4 ± 2.6	4.7	9.8 ± 1.9	0
> 100		No survivors			
Barley:					
0	20				93.1 ± 1.9
6.25 r/day	150	50.4 ± 0.6	—	17.9 ± 0.2	70.9 ± 0.8
11.0 „	110	49.7 ± 0.7	—	18.0 ± 0.3	67.7 ± 1.0
14.0 „	63	49.5 ± 0.9	—	17.2 ± 0.5	67.4 ± 1.4
19.0 „	66	52.0 ± 1.0	—	18.2 ± 0.8	63.6 ± 0.9
22.0 „	36	48.3 ± 1.2	—	13.3 ± 0.4	51.1 ± 1.8
27.0 „	43	55.6 ± 1.1	—	14.9 ± 0.4	53.7 ± 1.1
34.0 „	51	55.0 ± 0.9	—	14.7 ± 0.4	48.3 ± 2.1
36.0 „	51	63.8 ± 1.0	—	17.9 ± 0.3	34.6 ± 1.9
40.0 „	42	60.5 ± 1.0	—	16.2 ± 0.4	23.5 ± 2.4
45.0 „	38	61.0 ± 0.9	—	15.4 ± 0.4	15.0 ± 1.4
50.0 „	45	58.7 ± 0.8	—	15.3 ± 0.6	0.05
56.0 „	36	55.5 ± 0.8	—	13.7 ± 1.0	0.03
64.0 „	36	54.2 ± 0.9	—	15.4 ± 0.5	0.02
73.0 „	31	50.1 ± 1.1	—	—	0
> 73		No survivors			

the larger doses applied (Table 2). At a dose-rate of 100 r/day the plant height is reduced approximately 50 per cent. The lethal dose for oats seemed to be 100—120 r/day.

It appears from Table 2 that the number of straws per plant increased with increasing dosage. This finding is not due to a thinner stand with the higher doses. Distances between plants were equal at all levels of radiation exposures. In the barley no exact data on the number of straws per plant were obtained since a large number of straws had broken off during harvest and storage before the analyses were carried out. No figures, therefore, are listed in Table 2.

In oats the number of flowers per head was markedly reduced with larger doses. With plants exposed to a dose-rate of 100 r/day the number of flowers per head was reduced to one-third compared to the number of flowers on plants exposed to 12.5 r/day. In barley no effects of the chronic gamma radiation of doses from 6.25 to 64 r/day on the number of flowers per head could be demonstrated. Plants exposed to 73 r/day showed no normal development, and no head or seeds appeared on the plants. Between 6.25—64 r/day the number of flowers varied from 13.3 to 17.9. No systematic decrease with increasing dosage was demonstrated.

b. *Effects on fertility.* The chronic gamma radiation had pronounced effects on seed-setting in both oats and barley, indicating that fertility is markedly influenced by the radiation treatment (Table 2 and Figure 2).

The untreated controls of both oats and barley had a fertility of 95.2 and 93.1 per cent, respectively. The reduction increased with dosage but the slopes of dose curves seemed to be different for the two

TABLE 3. *Regression analyses of the effects of dose on fertility in oats and barley.*

Sources of variation	Barley (2x)				Oats (6x)			
	DF	SS	MS	F	DF	SS	MS	F
Linear regression	1	7695.22	7222.23	285.12***	1	5206.08	5206.08	141.48**
Excess due to quadratic	1	109.77	109.77	4.33	1	928.18	728.18	19.78*
Excess due to cubic	1	160.57	160.57	6.34*				
Residual	8	202.64	25.33		2	73.59	36.80	

species. Statistical analyses (Table 3) indicate that regression can be expressed by a quadratic equation in the case of oats, whereas the dose effect in barley is best described by a cubic regression curve.

III. DISCUSSION

When oats and barley are to be compared it is apparent that the neutron and chronic radiation treatments did not affect the two species in the same manner. When survival is taken as a criterion it is obvious that oats can survive larger doses of neutrons as well as chronic gamma radiations than barley. Oats did easily survive after being exposed to 3600 reps of neutrons while a dose of 1800 reps left no survivors in the barley variety, *Maja*. Oats survived a dose-rate of 100 r/day through 108 days whereas more than 75 r/day over the same period had an entirely lethal effect in barley.

In oats the retarding effects on growth are demonstrated by reduced plant height both after neutron and chronic gamma radiation exposures. The same is certainly true in barley although no effect of plant height in the surviving plants could be demonstrated by the neutron doses applied. Exposed to chronic gamma irradiation it seemed as if intermediate doses stimulated growth and larger doses reduced growth (Table 2). Stimulation on growth by weaker doses has been reported (SAX 1955, among others). In the material presented here the plants were grown at different distances from the source. Thus, randomization of treatments could not be arranged. The differences in plant height of barley exposed to 6.25—73.0 r/day may therefore rather be due to the soil heterogeniety than to the radiation treatments. Larger doses did certainly inhibit plant growth, but the plants did not reach maturity and were killed before the plants were fully developed.

The number of straws per plant was not influenced by the neutron treatments in oats and barley. Oat plants exposed to chronic gamma radiation, however, had an increasing number of straws. No analyses of tillering of barley were carried out.

The number of flowers per head in barley seemed to be unimpaired by both neutron and chronic gamma radiation exposures. In oats, however, both types of radiation reduced the number of flowers per head. The reduction increased with increasing dosage.

Fertility was impaired by radiation treatments in both oats and barley. After neutron treatments barley seemed to be more sensitive than oats. By studying the shapes of the fertility/dose effect curves

(Figure 1) it appeared that barley was about three times more sensitive than oats. Similar effects on fertility are also found in comparisons between wheat and barley exposed to neutron irradiation (MAC KEY 1952). It is interesting to note that the survival curves for X-irradiated diploid and polyploid yeast colonies (TOBIAS 1952) are in good agreement with the fertility curves for the diploid barley (2n=14) and the hexaploid oats (2n=42), indicating that different numbers of sensitive sites are present in the cells. If the sensitive sites are located in the chromosomes, the hexaploid oats should be more radio-resistant than barley, due to a larger (three times) number of chromosome complements.

The experimental results speak in favour of such a view. The reduction in fertility, which is obtained with 1200 reps in barley, required in oats 3600 reps, a three times stronger dose, to produce an equivalent effect. From the curve (Figure 1) it can also be demonstrated that a dose of 600 reps in barley produced the same reduction as would be expected with a dose of 1800 reps in oats. Thus, it seems as if the sensitivity of oats and barley to neutron irradiation is proportional to the number of chromosome complements. After chronic radiation, however, no such relationship seemed to exist. In this case oats proved to be more radio-sensitive than barley. This fact may indicate that the sensitivity to chronic gamma radiation is independent of the ploidy of the cell nucleus of the two species. The essential differences are illustrated by the slopes of the two curves (Figure 2).

The different responses of oats and barley to neutron and chronic gamma radiation indicate that neutron and chronic gamma radiation exert their effects through two different mechanisms. After neutron radiation the cyto-genetic effects were a more important factor than extragenic or physiological radiation injury. The effects of chronic gamma radiation, on the contrary, are probably due to extragenic injuries or physiological disturbances independent of the chromosome complements.

Whatever is the true nature of the effects of the two kinds of radiation, it may, however, be concluded that neutrons and chronic gamma radiation do not affect fertility in the same manner in the two species.

Since oats and barley are two quite different species, it would be of great importance to check these findings in more closely related forms. Experiments are, therefore, undertaken with diploids and autotetraploids of the barley variety, Brage.

SUMMARY

Seeds of hexaploid oats and diploid barley were irradiated in the centre of an atomic reactor and were exposed to various doses of neutrons of different energies. Plants of the same species were grown in the field during continuous exposure to gamma radiation.

The effects of these two types of radiation were studied on height of plants, number of straws per plant, number of flowers per head and fertility. The experimental results indicate that barley and oats were not affected to the same degree and in the same manner after exposure to neutrons and chronic gamma radiations.

Acknowledgement. — The authors wish to express their sincere thanks to K. STEENBERG for dosimetrical measurements of the gamma radiation.

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FREQUENCY DISTRIBUTIONS OF TOTAL FINGER RIDGE COUNTS IN THE SWEDISH POPULATION

By *J. A. BÖÖK*
STATE INSTITUTE FOR HUMAN GENETICS, UPPSALA, SWEDEN
(Received January 26th, 1957)

INTRODUCTION

SINCE modern physical anthropology has adopted the methods of population genetics it is important to extend the investigations on genetically determined normal traits. Among those the dermal patterns are very useful. A great deal of work has been done on finger prints since 1823 (PURKINJE). The comparisons between different populations have been based, almost exclusively, on a qualitative analysis of pattern types. Such an analysis does not easily lead itself to genetical interpretations. In spite of some deficiencies (cf. CUMMINS and MIDLO, 1943) the total ridge count has proved a suitable quantitative trait for genetical analyses. It measures the pattern size. The use of the actual ridge count is more convenient than the »quantitative value» as defined by BONNEVIE (1924).

So far no data on finger prints from the Swedish population have been published. The present data have been collected to contribute to the anthropology of this population and to be further analysed in order to evaluate their possible usefulness in investigations of disputed fatherhood.

In a series of papers HOLT (1949, 1952, 1953, 1954, 1955, 1956) has shown that pattern size as measured by the total ridge count is inherited. Dominance is absent and the effect of the alleles concerned is additive. No appreciable environmental (maternal) effect was found. Consequently it should be of some importance to compare the distributions of total ridge counts in different populations. So far we have comparable information from England, France and Portugal (Table 1).

TABLE 1. *Means and standard deviations of total ridge counts in four surveys.*

Place and source	Males			Females		
	No.	Mean	S. D.	No.	Mean	S. D.
Great Britain (HOLT, 1955)	825	144.98	51.08	825	127.23	52.51
Portugal (DA CUNHA a. ABREV, 1954)	100	140.5	42	100	126.3	46
France (LAMY et al., 1956)	351	132.36	45.28	360	121.36	46.48
Sweden (present data)	204	139.70	49.47	188	120.67	52.81

METHOD

The finger prints were recorded according to a slight modification of the method described by BÖÖK (1948). After cleaning the fingers the pattern area was rubbed lightly with a small amount of lampblack. A piece of commercial Scotch tape was placed on the pattern. The strip was then removed gently and attached to a sheet of paper. The method makes possible also the analysis of fingerprints from children, in general from the age of 2—3 months.

THE PRESENT DATA

The material consists of 100 families with both parents and one child (Table 2). In 10 families two children have been examined. All these individuals have been subjected to thorough anthropological examinations which included the recording of finger prints. The families were obtained through their children who were registered at the Children's Health Centers in Uppsala. All individuals are of Swedish extraction and all social categories are represented. Although all families were resident in Uppsala the birthplaces of the parent were distributed all over the country. About 30 per cent of the parents were born in the county of Uppsala. These data were collected primarily to be used for a critical evaluation of the possibilities of using different anthropological examinations in cases of disputed fatherhood.

Further finger prints were taken from 29 cases of disputed father-

TABLE 2. *Distribution of total finger ridge counts in 100 Swedish families.*

Family no.	Parents		Children		Family no.	Parents		Children	
	Father	Mother	Males	Females		Father	Mother	Males	Females
1	225	54	—	171	43	161	118	89	—
2	132	90	—	81	44	248	7	—	83, 163
3	131	119	—	119	45	126	151	145	—
4	213	84	—	—	46	191	127	—	135
5	102	107	47	—	47	139	174	162	—
6	211	50	—	90	48	214	256	—	185
7	160	4	—	—	49	134	137	100	—
8	91	179	—	218	50	91	128	80	—
9	87	162	152	—	51	213	85	—	120
10	103	119	—	138	52	176	229	—	248
11	132	11	—	160	53	143	71	—	106
12	141	148	—	147	54	35	—	—	—
13	204	140	86	—	55	214	171	—	185
14	105	55	—	81	56	98	99	34	—
15	72	146	—	104, 123	57	228	167	120	—
16	166	150	179, 185	—	58	52	84	34, 83	—
17	88	33	18	—	59	26	216	—	47
18	242	136	239	—	60	117	216	175	153
19	228	125	179	169	61	135	213	221	—
20	78	175	—	75	62	168	118	160	—
21	163	156	180	—	63	184	146	—	145
22	157	132	164	—	64	203	144	177, 188	—
23	160	177	—	182	65	208	62	—	93
24	159	125	—	134	66	149	115	76	—
25	189	134	—	192	67	87	164	96	—
26	178	187	—	—	68	200	120	180	—
27	148	144	144	—	69	155	56	—	39
28	233	174	104	—	70	101	82	—	38
29	148	86	120	—	71	81	149	133	—
30	40	162	—	22	72	134	123	—	185
31	161	138	146	—	73	106	171	—	164
32	172	115	—	—	74	147	191	—	106
33	201	140	—	115	75	105	164	—	81
34	190	167	190	—	76	211	32	—	81
35	165	43	78	—	77	122	173	—	171
36	146	150	151	—	78	84	98	59	—
37	91	111	—	24	79	210	134	125	—
38	87	83	160	—	80	93	5	95	—
39	171	25	—	112	81	183	113	—	139
40	169	258	128	—	82	158	197	212	—
41	147	121	—	129	83	178	60	136	—
42	176	137	—	195	84	64	86	141	60

Family no.	Parents		Children		Family no.	Parents		Children	
	Father	Mother	Males	Females		Father	Mother	Males	Females
85	201	156	—	111	93	167	115	—	173
86	44	156	112	—	94	112	71	—	146
87	203	60	178	—	95	162	130	112	—
88	176	114	171, 142	—	96	165	117	—	121
89	169	129	179	—	97	185	192	—	189, 144
90	74	—	54	—	98	153	84	—	62
91	195	14	—	73	99	185	88	171	—
92	160	98	—	20	100	185	60	—	167

TABLE 3. *Distribution of total finger ridge counts in 29 cases of »disputed paternity».*

No.	Mother	Child		»Fathers»
		Males	Females	
1	120	142	—	185, 151
2	106	—	176	132 —
3	—	213	—	116, 181
4	58	86	—	181 —
5	107	—	127	114 —
6	115	139	—	126 —
7	132	—	—	— —
8	98	102	—	68 —
9	205	142	—	123 —
10	91	138	—	101, 198
11	229	199	—	167 —
12	158	158	—	47 —
13	11	—	5	71 —
14	89	—	95	175 —
15	97	137	—	124 —
16	101	132	—	95 —
17	207	—	171	— —
18	136	140	—	165, 125
19	28	—	152	171, 109
20	61	—	128	151, 98
21	139	71	—	43 —
22	88	—	68	87 —
23	35	98	—	129 —
24	79	—	149	125, 113
25	110	85	—	91 —
26	71	103	—	104, 107
27	68	131	—	149 —
28	108	—	—	129, 31
29	139	178	—	— —

TABLE 4. *Survey of the present data.*

Type of sample	Parents		Children		Total
	Fathers	Mothers	Males	Females	
100 »normal» families	100	98	51	53	302
29 cases of disputed paternity	35	28	18	9	90
Total	135	126	69	62	392

hood handled by the author during 1951—55 (Table 3). Here we have prints from the mother, one child and one or two possible fathers. The residences and birth places of these individuals were scattered all over Sweden.

A few finger prints had to be discarded due to technical deficiencies. After this selection there remained for analysis prints of 392 individuals (204 males and 188 females). Table 4 gives a survey of the sample according to sex and familial relation. The actual total ridge counts have been given in full in Tables 2 and 3.

DISTRIBUTIONS

The distributions for males and females are given in histograms in Figs. 1 and 2. The range is 18—248 for males and 4—258 for females. The modal class is at 160—179 ridges for males and 120—139 ridges for females. The difference between the two means, 139.7 ± 3.5 for males and 120.7 ± 3.9 for females, is significant.

Means and standard deviations have been calculated also for »all fathers», »all mothers», »all sons» and »all daughters» and are given in Table 5 together with the differences (and their significances by the t-test) between father-mothers, sons-daughters, fathers-sons and mothers-daughters. Skewness and kurtosis have been calculated (using a group interval of 20) for males and females by the method of FISHER (1954).

The values of g_1 (skewness) and g_2 (kurtosis) are:

$g_1 = -0.24 \pm 0.17$ and $g_2 = -0.48 \pm 0.34$ for males
$g_1 = -0.07 \pm 0.18$ and $g_2 = -0.29 \pm 0.35$ for females.

Both distributions are negatively skew and flattened but no value is significant.

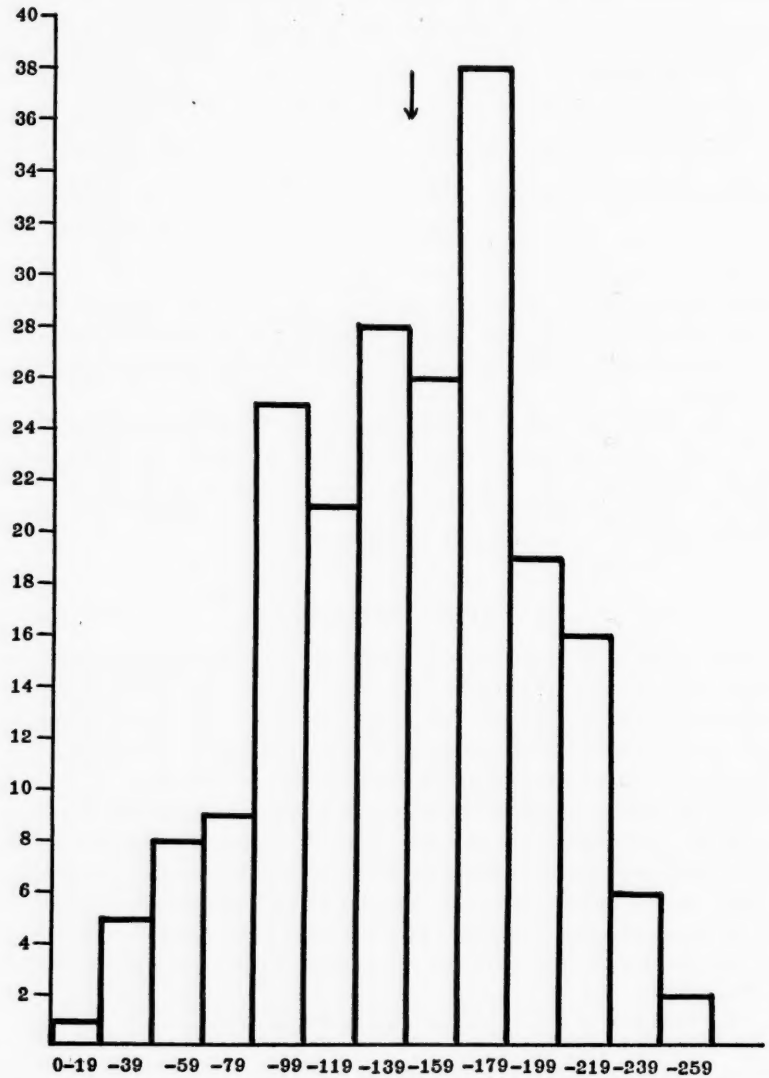

Fig. 1. Total ridge count distribution (with grouping interval 20) for 204 males. The arrow indicates the mean.

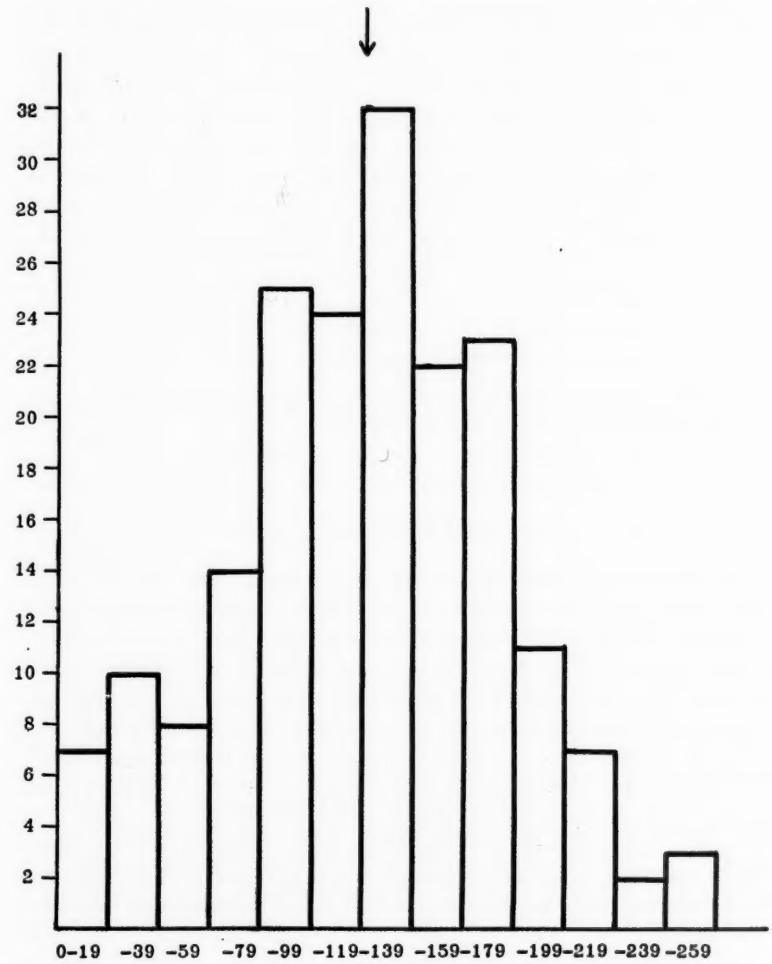

Fig. 2. Total ridge count distribution (with grouping interval 20) for 188 females. The arrow indicates the mean.

TABLE 5. *Means and standard deviations of components of the groups according to sex and family place, and significance of their differences.*

Family place	Mean and S. R.	S. D.	
Fathers	142.72 ± 4.29	49.83	
Mothers	119.42 ± 4.72	53.67	
Sons	131.75 ± 5.80	48.18	
Daughters	123.37 ± 6.71	52.86	
Difference between	**Difference and its standard error**	**t**	**D. F.**
Fathers—mothers	23.30 ± 6.38	3.65	259
Sons—daughters	8.38 ± 8.87	0.94	129
Fathers—sons	12.94 ± 7.02	1.84	50*
Mothers—daughters	— 9.16 ± 8.06	1.14	62**

* 47 fathers (exclusive of fathers in Table 3); of these 4 had each 2 sons.
** 60 mothers; of these 3 had each 2 daughters.

COMMENTS

The mean ridge values of this sample are lower than those found by HOLT (1955) in England and by XAVIER DA CUNHA and ARAUJO ABREU (1955) in Portugal but higher than those of LAMY *et al.* (1956) in France. There are, however, no clear-cut differences between the four samples. The only significant difference is between the English and French males, *i. e.* 12.6 ridges with a standard error of 3.0 ridges.

The distributions in the present sample do not deviate significantly from normality. This suggests that a large number of additive genes are involved. Also the distributions of the French sample (LAMY *et al.* 1956) are negatively skew and flattened but not significantly. HOLT (1955), however, in her larger sample found non-gaussian distributions, suggesting that only a small number of genes with appreciable effect were involved.

Comparing the present data with those collected in other countries one may conclude that among the samples from four different European populations no clear-cut differences in pattern size, as measured by total ridge counts, can be demonstrated.

Acknowledgement. — The present investigation was aided by a grant from the Swedish Medical Research Council.

SUMMARY

Total finger ridge counts from a sample of the Swedish population have been analyzed. The mean values are 139.7 (S. D.=49.5) for males (204 individuals) and 120.7 (S. D.=52.8) for females (188 individuals). The difference between the two sexes is significant. The distributions are negatively skew and flattened, but no value of skewness or kurtosis as calculated by FISHER's method is significant. The differences observed among the values for Sweden and those from 3 other European countries are not significant. Total ridge count is a suitable quantitative character for genetical and population studies.

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POST-IRRADIATION EFFECTS ON X-RAY-INDUCED MUTATION IN BARLEY SEEDS

By *L. EHRENBERG* and *U. LUNDQVIST*
INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY, UNIVERSITY OF STOCKHOLM, AND INSTITUTE OF GENETICS, UNIVERSITY OF LUND
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INTRODUCTION

IT has long been accepted that biological injury caused by ionizing radiations can be modified by factors applied after the irradiation. Chromosomal aberrations have been especially well studied in this respect. Although several points are still a matter of controversy, there are data indicating that the breakage process is slow and can be influenced (*cf.* SWANSON, 1954), and others showing that the rate of a reunion of already broken ends can be changed (*cf.* WOLFF and LUIPPOLD, 1955). It is also well established that, in different organisms, radiation-induced physiological damage, *e. g.*, growth inhibition and lethal effects, can be influenced by treatments after irradiation. (In many cases such effects are more or less a function of chromosome damage.) In contrast, data are less numerous for a modification after the irradiation of gene mutation: HOLLAENDER (1956; *cf.* HOLLAENDER and KIMBALL, 1956) presented indications of a decrease of the frequency of X-ray-induced mutations in *Escherichia coli*, provoked by a post-irradiation treatment with certain metabolites; and KIMBALL (1955) observed a similar effect in *Paramecium* after treatment with hydrogen peroxide.

In plant seeds it seems *a priori* possible to change mutation rates by a variation of the conditions after irradiation. In this rather dry tissue the extent of radiation injuries of different kinds slowly grows during storage after treatment (*cf.* GUSTAFSSON, 1947; EHRENBERG, 1954, 1955; ADAMS *et al.*, 1955), and strong indications have been obtained that mutation rates do not behave differently in this respect (unpubl.). Thus, the evidence points to a protracted lability of the primary radiation effects.

The aim of the present investigation was to study whether mutation induced in seeds of a higher plant, barley, could be modified by factors applied during germination. Besides theoretical aspects this question is also of practical interest: In plant breeding by means of radiation-induced mutation an enormous variation is obtained in mutation rate, survival, and other effects. Could this variation be controlled by a standardized treatment of the material, the breeders' task would be appreciably simplified.

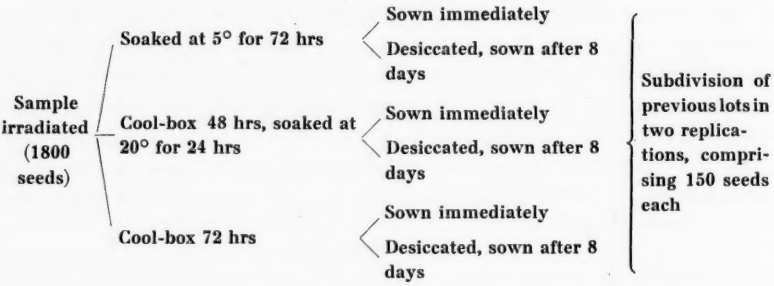
Some of the factors which cause this variation act during irradiation. The water content of the seeds, especially, seems to determine the degree of injury of all kinds, including survival, sterility, and mutation rate in field experiments (EHRENBERG, 1955 a).

In the paper quoted were found obscure side-effects of the storage time before sowing and of the temperature during germination. In laboratory experiments, where irradiated resting seeds (< 25 % H_2O) were allowed to absorb water up to about 35 % (where germinative processes are initiated) at different temperatures, the frequency of chromosomal aberrations (GELIN, 1953) and the degree of growth inhibition (EHRENBERG, 1955 b, c) were about 50 % higher at lower temperatures (5—12° C.) as compared with higher ones (20—30°). This water uptake occurs during the first 24 hours' soaking on wet filter paper at 20° C. During later stages of germination the material is much less, if at all, influenced by temperature: The phenomenon could be described as a stabilization of the earlier labile injury when water has been absorbed up to a certain critical concentration.

METHODS

Resting seeds of Bonus barley were irradiated with 175 kV unfiltered X-rays (1000 r/min.). Seeds containing 11.3 % and 14.7 % water were used. (For details of the techniques, see EHRENBERG 1955 a.)

It was desired at a given moment to dispose of identically irradiated and correspondingly developed seed samples which had been soaked at different temperatures. The material was not allowed to differ as to post-irradiation storage effects. Each irradiated sample was therefore subdivided as follows (one unsoaked sample was always studied simultaneously):


Sample irradiated (1800 seeds)
Soaked at 5° for 72 hrs
Sown immediately
Desiccated, sown after 8 days
Cool-box 48 hrs, soaked at 20° for 24 hrs
Sown immediately
Desiccated, sown after 8 days
Cool-box 72 hrs
Sown immediately
Desiccated, sown after 8 days
Subdivision of previous lots in two replications, comprising 150 seeds each

Immediately after irradiation each seed sample was divided into three parts. The first was soaked for 72 hours at 5° C., the second was stored in the cool box at — 20° for 48 hours and then soaked at +20° for 24 hours and the third part was left for 72 hours in the cool-box. From previous experiments 72 hours' soaking at 5° C. was known to correspond to 24 hours at 20° in bringing the seeds past that stage where germination temperature exerted its influence. The effect of storage on radiation damage was totally eliminated at — 20° C.

Of each of the three samples one half was sown in the field immediately after soaking, the other half after re-desiccation and storage at 20° for one week. (From laboratory experiments involving measurement of the growth rate of seedlings, it was known that irradiated seeds, once soaked and re-desiccated, became »locked up» as to radiation damage, *i. e.*, they were not affected by storage effects nor by germination temperature. The re-desiccation and later sowing in the present case was done in order to test whether, by such a treatment, the material could be standardized, *i. e.*, made independent of all the conditions causing a variation between experiments. Although the results do not contradict this possibility, all the samples sown later, including the unirradiated controls, suffered under bad germination conditions which so injured the plants as to totally change the results; *v. infra.*) In the last instance the material was subdivided into two replications sown in different parts of the field. No differences were found between the replications, and these could therefore be lumped in most treatments.

The analyses of spike fertility, mutation rate, seed germination, and plant survival were made as described in 1955 a.

RESULTS

The experimental data are summarized in Figs. 1—4 and Tables 1—2.

The *survival*, which well reflects the seed germination, was left unimpaired by the doses, 2—6 kr, used at the lower water content. At 14.7 % H_2O a distinct decrease was obtained with 15 kr (*cf.* Fig. 1), especially at the lower germination temperature ($p < 0.001$ for the temperature effect at this dose). In the material sown later there was, already in the control materials and in the unsoaked series, an additional fatality of about 20 % of the plants. (At low doses and in the controls — *cf.* Fig. 1 — this additional lethality was highest after soaking at the higher temperature.) As regards developed lethality, the unsoaked series behaved exactly as the one soaked at 20° C.

The *sterilities* induced, expressed as per cent of sterile flowers per spike, were determined as $S=100\ (F_C - F_{irr})/F_C$, F_C being the average fertility of spikes of a control sample, and F_{irr} that of an irradiated sample. (At the lower water content, F_C amounted to 98.1 and at the higher content to 98.8 %.)

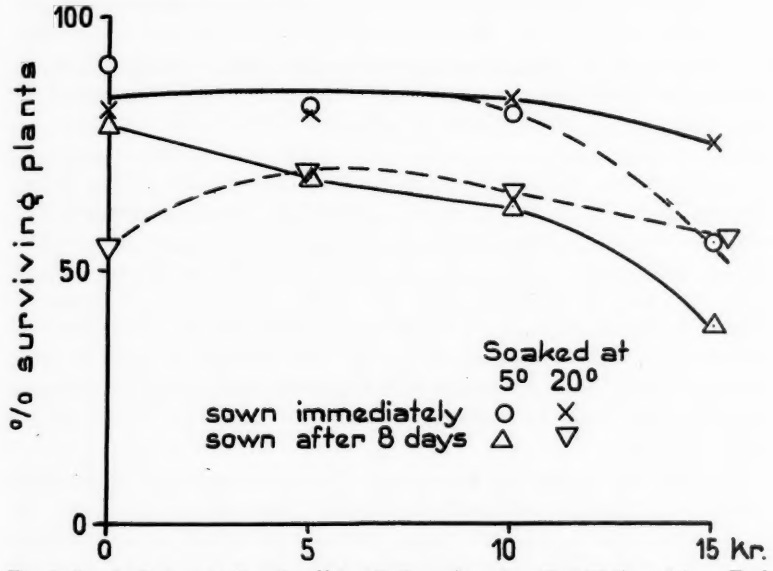


Fig. 1. Survival at maturity after X-irradiation of seeds with 14.7 % moisture. Each point represents 300 seeds sown.

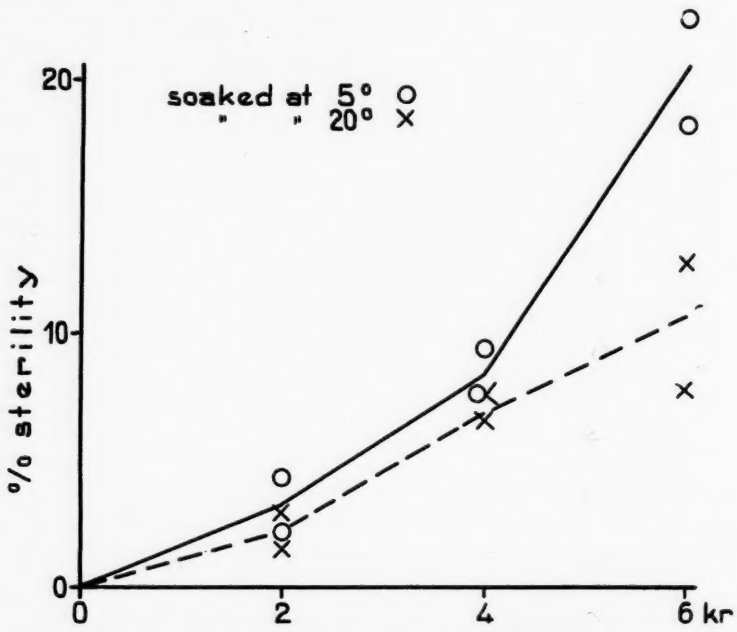


Fig. 2. Sterility after irradiation of seeds with 11.3 % moisture. Sowing immediately after soaking at different temperatures. Data for replications given individually (each point averages about 100 analyzed spikes).

At both water contents the lower soaking temperature gave a higher sterility (Figs. 2, 3). A test of the significance of this difference was made as in 1955 a, the differences $d = \log S_{5°} - \log S_{20°}$ being determined. The same average value $\bar{d}_S = 0.179 \pm 0.037$, was found in both series. A *t*-test of the value gives $p < 0.001$ (11 degrees of freedom). This $\bar{d}$ corresponds to a 51 % higher sterility at the lower soaking temperature. The unsoaked series take an average position between the two soaked series irradiated at the same water content. In all series the effectiveness of the radiation is about 25 % smaller at the higher water content.

The *mutation rates* induced, which were determined as frequencies per spike progeny of chlorophyll deficient mutants, are given in Fig. 4, *cf.* also Table 1. At both water contents the lower soaking temperature has about the same enhancing effect on the mutation rate. On an average, the difference, $\bar{d}_M$, between the logarithms of mutation

TABLE 1. *Average mutation frequencies per r and per spike progeny, $\times 10^6$, and number of spikes developed per plant after different treatments.*

Soaking conditions	Sowing conditions	Moisture content of seeds			
		11.3 %		14.7 %	
		mut. rate	spikes/plant	mut. rate	spikes/plant
Soaking at 5°	Immediate sowing	8.8	5.30	5.9	4.65
Soaking at 20°		7.1	4.91	4.8	4.77
No soaking		6.3	4.82	5.7	4.50
Soaking at 5°	Sowing after 8 days	6.9	3.58	3.6	2.90
Soaking at 20°		4.9	3.36	3.7	3.23
No soaking		5.1	3.41	3.4	2.90

rates amounts to 0.090 ± 0.021. For 5 degrees of freedom this gives $t=4.3$, $0.01 > p > 0.001$. The difference, corresponding to a 23 % higher rate at the lower temperature, can therefore be regarded as real. The different types of chlorophyll mutations, grouped as *albina*, *viridis* — *i. e.*, the two most common types — and others, seem to be affected equally by the soaking temperature (Table 2, upper half).

The difference, 23 %, between the soaking temperatures, seems smaller than the value, 51 %, obtained for the corresponding differences between sterilities. A *t*-test of the difference between the log ratios,

TABLE 2. *Influence of soaking temperature and later sowing on distribution of mutation types.*

Soaking conditions	Sowing conditions	Mutation type						Total number
		albina		*viridis*		others		
		number	%	number	%	number	%	
Soaking at 5°	Immediate sowing	126	39.0	156	48.3	41	12.7	323
Soaking at 20°		106	39.8	119	44.7	41	15.5	266
No soaking		127	39.9	143	45.0	48	15.1	318
Soaking at 5°	Sowing after 8 days	49	51.0	39	40.6	8	8.3	96
Soaking at 20°		50	43.8	46	40.3	18	15.8	114
No soaking		48	50.0	30	31.2	18	18.8	96

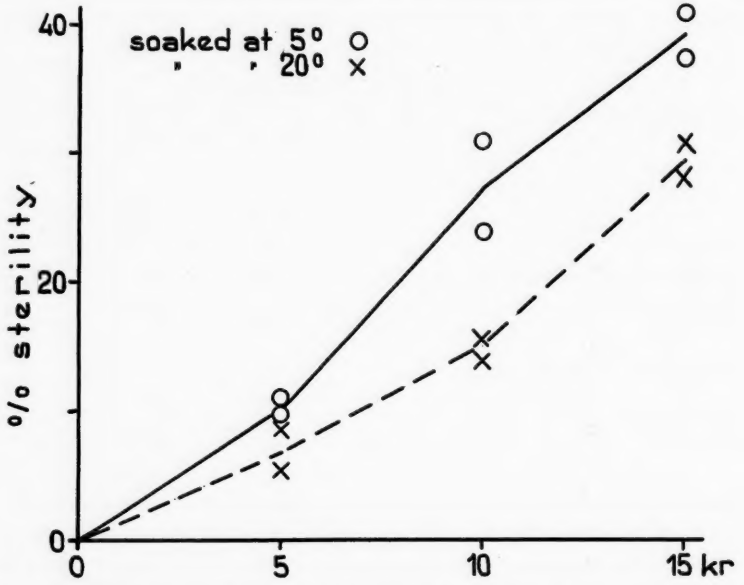


Fig. 3. Sterility after irradiation of seeds with 14.7 % moisture. Otherwise as Fig. 2.

$\bar{d}_S - \bar{d}_M = 0.087 \pm 0.041$ gives $p \sim 0.05$. It can therefore be said that the material is too small to permit the statement that the soaking temperature affects sterility and mutation differently. This is the more true, given the fact that the two measured properties are not independent of one another. At higher sterilities the chance of loosing mutation cases increases (*i. e.*, with fewer fertile flowers per spike the recessive homozygote has a lower probability of realization). This interrelation would mean that the measured temperature effect is smaller than the one really induced.

The influence of the water content at irradiation on the mutation rates seems large in the soaked series: an almost 50 per cent higher rate is obtained at 11.3 %, as compared with 14.7 % H_2O (Table 1). In the unsoaked series the difference amounts to only 10 per cent, which is more in accordance with earlier results (EHRENBERG, 1955 a). As a matter of fact the dry, unsoaked series (mutation rate $6.3 \cdot 10^{-6}$) is similar to the one soaked at 20° (mutation rate $7.1 \cdot 10^{-6}$) whereas the unsoaked seeds, irradiated at a higher water content ($5.7 \cdot 10^{-6}$ mutations), are more equal to those soaked at a low temperature ($5.9 \cdot 10^{-6}$ mutations). If these relationships are real, they cannot be explained at present.

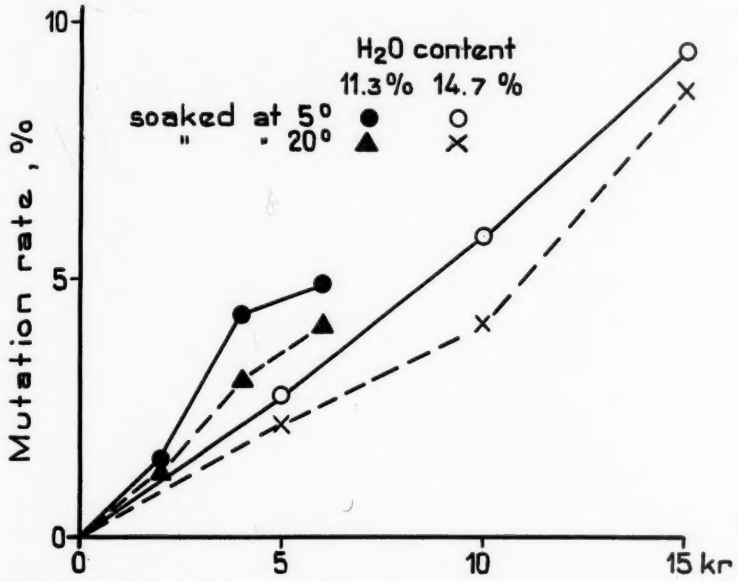


Fig. 4. Mutation rates, per spike progeny, after irradiation of seeds containing 11.3 and 14.7 % water, respectively, then soaked at 5 or 20° C. Sown immediately after the end of soaking. Each point represents about 1200 analyzed spike progenies.

In the *material stored* for eight days the mutation frequencies were throughout about 30 per cent lower, as compared with the immediately sown series (*cf.* Table 1). A *t*-test, performed as in the analyses of sterility and mutation above, gives $p \sim 0.001$; the difference is without doubt real. This decrease of mutation cases is certainly dependent on certain conditions causing a bad development of the plants: The lower survival rate has already been mentioned (p. 393). The number of spikes per plant was reduced by about 1.5 (Table 1). The relative sterility *S* (p. 393) was not significantly changed, however, when a 3—5 % lower fertility in the unirradiated control series is considered. The bad development is not provoked by the soaking-desiccation treatment, since it is already found in the unsoaked series.

DISCUSSION

In the present study it is shown that effects, observed at a late stage of development (the mature plant or the following generations) and

induced by irradiating the resting barley seed, can be influenced during the germination of the seed. When germination occurs at a low temperature (5°) the sterility was found to be 51 % higher and the mutation rate 23 % higher as compared with data from otherwise equally treated samples allowed to germinate at 20° C. Although the sterility seems more affected than the mutation rate, this difference cannot be looked upon as certain. The chief reason for this is the fact that the two properties are not independent of each other, since an increased sterility causes a loss of mutation cases. The germination temperature also exerted a similar influence on the survival rate at high doses.

In his studies of the first mitotic division in the roots of germinating seeds, GELIN (*l. c.*) found about the same temperature influence as is now demonstrated for sterility. Since sterility is chiefly caused by gross chromosomal rearrangements, it is to be expected that the later stage, in a simple manner, reflects the earlier one. It is obvious, however, that under certain hitherto undefined conditions the agreement might be disturbed by eliminatory processes which are also influenced by germination temperature, though possibly at a later developmental stage (*cf.* EHRENBERG, 1955 a).

Growth inhibition, too, was found to be about 50 % greater at a lower germination temperature (EHRENBERG, 1955 b). Although the causes of radiation-induced growth inhibition, as well as lethality, are still somewhat obscure, an interrelation with chromosomal aberrations certainly exists, especially at high doses, where the inability of meristematic cells to divide becomes a factor of major importance.

The mutations studied, and which can be modified in a manner similar to phenomena more or less related to chromosomal aberrations, are rather to be described as changes in genes, »point» mutations. As regarded from the linearity with dose, clearly manifested in large series at low doses (*cf.* EHRENBERG, 1955 a), the mutations are induced in true one-hit events. In contrast, sterility depends exponentially on the dose.

A mutation rate has always to be expressed as the number of mutated units (individuals, *etc.*) per total number of units investigated, where, in the ideal case, the latter number should be equal to or stand in some simple relation to the number of irradiated cells, the progenies of which are studied. A repair from mutation, measured as a decrease of the percentage of mutated units, could now, in principle, be brought about in different manners: As a true repair we would define a case

where the mutated gene is reverted to the original, non-mutated, state under certain conditions applied after the moment of energy absorption. Such a case would indicate a slowness in the changes leading to mutation, possibly a lability for some time of a directly hit gene. If, on the other hand, the post-irradiation treatment leads to an increased elimination (lethality) of cells — and preferably those containing a mutation — a repair is simulated. This possibility makes it difficult to interpret mutation experiments with multicellular as well as unicellular organisms. Still more complicated is the situation where the mutated cells develop more slowly than the non-mutated ones, or when, in a multicellular organism, the post-irradiation treatment promotes the replacement of mutated gametes or of cells from which the latter originate, by non-mutated neighbours. Modification of radiation damage in general is known to occur along these different lines.

Under the cultivation conditions used, a barley seed gives rise to a plant with about five spikes, each containing at maturity about 30 seeds which, in the case of heterozygosity segregate in the 3:1 ratio. When irradiation of resting seeds leads to induction of a mutation, one spike becomes heterozygous for the latter, in about 95 % of the cases, *i. e.*, at the moment of irradiation the mutating unit is the primordium cell of a spike. (In the remaining 5 % of the cases two or more spikes have had a common primordial cell at the moment of irradiation.) Several processes are known that lead to an elimination of genetically more damaged cells, cell groups, or individuals (*cf.* EHRENBERG *et al.*, 1953). Such factors play a role especially at high doses, where after X-irradiation a conspicuous drop in mutation frequency occurs as a function of sterility or lethality (this elimination of whole individuals hits the ones which are the most sensitive to lethal effects as well as to induced mutation). In some cases this drop is a consequence of an elimination of the most sensitive spikes, leading to a reduction in spike number.

In the present case the lower mutation rate obtained in the material sown later is obviously connected with the reduction in spike number. Probably the first developed spikes were most sensitive to induced mutation as well as to the bad field conditions leading to the injury. Under some hitherto undefined conditions the elimination of the top spikes at high doses is followed by an intense secondary tillering, a high number of secondary adventivous shoots with a low mutation frequency being formed (*cf.* MAC KEY, 1951). The latter case is thus a sort of replacement of damaged cells by others, as occurs in treatment

with bone marrow cells after total-body irradiation of mammals (*cf.* FORD *et al.*, 1956).

The present effect of the germination temperature cannot be explained on the basis of a greater elimination of mutated units at the higher temperature, since the number of spikes per plant is not changed (Table 2) and since eliminating principles such as lethality and sterility work in the opposite direction. Furthermore, since the growth inhibition is less pronounced at the higher temperature, it is not probable that any cells are more effectively removed here. There remains, of course, a theoretical possibility (the demonstration of which will be an intricate embryological problem) that radiation-injured cells are more effectively substituted by neighbour cells at the higher temperature. At present no circumstances speak in favour of such an interpretation. Most probably, therefore, the phenomenon should be explained as a true repair of the mutated genes. This is certainly the true explanation of the chromosomal aberrations and, consequently, the sterility. According to GELIN (personal communication; *cf.* also 1953, Tables) the number of cells entering the first mitosis is not affected by temperature, and consequently the difference in aberration frequency must be due to an after-effect on more or less latently broken chromosomes.

It is interesting to note that germination temperature affects different mutation types about equally, whereas the *viridis* mutations suffer more than the *albinae* under the eliminatory influences that were operating in the samples sown later. In some manner the *virides* appear bound to sterility: For example, at low and medium doses their relative frequency is found to increase under conditions favouring a high sterility. (This does not seem valid for the temperature effect, however, only a slightly higher value being found in the more sterile 5° series; *cf.* Table 2.) At very high doses, however, where a strong elimination is at work, the relative frequency of *virides* again drops (*cf.* EHRENBERG *et al.*, 1956). The fact, here again manifested, that one mutation type is more influenced than others by eliminating factors, involves a certain danger in experiments aiming at a directing of the mutation process. The possibilities of secondary eliminatory processes must be carefully considered before an agent is claimed to be specifically mutagenic.

The following effects of an X-irradiation of resting barley seeds are now known to become greater when the seeds are allowed to take up water at a lower temperature:

(1) frequency of chromosome aberrations in the first cell divisions;

(2) sterility;
(3) retardation of seedling growth;
(4) lethality, measured as a decreased survival of mature plants;
(5) gene mutation;
(6) retardation of the formation of α-amylase (EHRENBERG, 1955 c).

Apart from the relationships of (2)—(4) with (1), as discussed above, it is yet impossible to state how far the different effects are related or whether they have a common basic mechanism. WOLFF and LUIPPOLD (1955) have delivered data to show that factors — *e. g.*, KCN, CO in the dark, and also a lowered temperature — impairing oxidative metabolism prolong the »standing open» of chromosome breaks, *i. e.*, inhibit reunion. In our study of growth retardation (1955 b, c) quantitatively the same temperature effect was found in nitrogen and air. In the former gas the seeds merely swell without any sign of initiation of germination. This was taken to indicate that the temperature effect was probably not to be referred to a slowed-down (oxidative) metabolism. In view of the fact that WOLFF finds very low oxygen tensions to suffice for the maintenance of a normal reunion rate, the role of the metabolic rate has to be reinvestigated, use being made of perfectly oxygen-free nitrogen.

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SUMMARY

(1) Resting barley seeds, after irradiation by X-rays, show a higher rate of mutation as well as a greater sterility when made to germinate at 5° as compared to 20°. This holds equally for various types of mutations.

(2) The results reported make it seem highly probable that the effect of temperature on the X-ray-induced rate of point mutation involves a modification of primary injury, and should not be explained by elimination of mutated cells at the higher temperature. This finding agrees satisfactorily with investigations on chromosome aberrations and, consequently, sterility, which are, of course, more accessible to direct observation.

(3) Under conditions of bad growth leading to a decrease in the number of spikes per plant, the mutation rate becomes lower, due to prevalent elimination of mutated units. *Viridis* mutations suffer more than other types from this elimination.

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THE INHERITANCE OF THE ACCESSORY CHROMOSOMES IN CENTAUREA SCABIOSA

By *SUNE FRÖST*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN
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The present report deals with the transmission of the accessory chromosomes in various cross combination in *Centaurea scabiosa*. Most of the crosses made elucidate the inheritance of the standard type of accessory chromosomes but some crosses were also made to investigate the behaviour of the accessory iso-chromosomes (cf. FRÖST, 1956). Some of the data presented in this report have been published earlier (data given by FRÖST in MÜNTZING, 1954).

I. MATERIAL AND METHODS

All the plants used in these crosses originate from natural population of *Centaurea scabiosa*, and have been mainly collected in different parts of southern Sweden, Denmark and England. As *C. scabiosa* is an insect-pollinating species, manual pollination was undertaken in making the crosses. The method used here was as follows (cf. MARSDEN-JONES and TURRILL, 1937): before flowering the heads were isolated in pollen proof bags, brushed off and the pollen gathered in a bowl. The required pollen was then applied with a brush to the receptive stigmatic surfaces. This process was repeated every second day during the flowering time. A great number of heads were isolated and pollinated in every cross combination. The seed-setting has generally been satisfactory with this method. In some cross combinations, however, the seed-setting has been very low or has completely failed. The bad seed-setting in these cases probably resulted from incompatibility since *C. scabiosa* demonstrates a varying degree of self-sterility. When a plant is self-pollinated, the seed-setting is usually limited to a few seeds per head. In some cases, however, the seed-setting was considerable when the plants were being self-pollinated. To avoid the possibility of disturbing

effects from self-fertilization the cross combinations were choosen in such a way that the two parental plants had in each case the same number of accessories. The disadvantage of this type of cross is that an eventual difference in the transmission of the accessories between the male and the female side is impossible to detect. To investigate whether there were such differences a number of male-sterile plants were used.

II. THE INHERITANCE OF THE ACCESSORY CHROMOSOME OF STANDARD TYPE

1. Crosses of the type (20+1 st. acc.chr.)×(20+1 st. acc.chr.)

If it is assumed that both the parental plants produce gametes with one and without accessory chromosomes in equal frequencies then the F_1 plants should be expected to receive the following number of accessory chromosomes: 0, 1 and 2 in the relative frequencies: 1:2:1. It is, however, obvious from the chromosome numbers among the daughter-plants in Table 1 that these ideal ratios were not realized in the experiments.

The most obvious deviation is that there is an excess of plants without accessories in relation to plants with one and with two accessories. There is, consequently, a certain loss of accessories when they are transmitted from one generation to the next. The amount of this elimination is clear from a study of the mean values of accessory chromosomes for the different progenies (Table 1). In 16 out of 19 crosses the mean value of accessory chromosomes is lower for the F_1 plants than for the parental plants. The numerical decrease of the accessory chromosomes from one generation to another in *C. scabiosa* is very probably caused by a certain degree of meiotic elimination of the accessories (FRÖST, 1956). In several grass-species it has been reported that the accessory chromosomes are furnished with a special mechanism of non-disjunction which causes the accessories to maintain or even to increase their number from one generation to another in spite of a meiotic elimination. In *C. scabiosa*, however, there does not seem to exist a mechanism for numerical increase of the accessories sufficient to counterbalance their meiotic elimination.

There are some plants (Table 1) with 3 accessory chromosomes which show that a number of gametes must have received at least 2 accessory chromosomes. In order to obtain an idea of the relative

TABLE 1. *Number of accessory chromosomes resulting from crosses of the type (20+1st. acc.)×(20+1st. acc.).*

Parents		F_1					Number of plants	$\bar{x}$ acc. chr. per plant
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1	2	3	4		
3b—18 × 3b—5	1 × 1	11	14	6			31	0.84
14b—11 × 3b—7	1 × 1	34	32	10	1		77	0.71
3b—7 × 14b—11	1 × 1	9	16	9			34	1.00
3b—5 × 8b—18	1 × 1	13	22	16	6		57	1.26
8b—18 × 3b—5	1 × 1	25	24	16			65	0.86
*D—8—14 × D—14—8	1 × 1	23	26	8	2		59	0.81
*D—8—14 × S—60—12	1 × 1	44	29	13	3		89	0.72
*D—8—14 × S—61—8	1 × 1	34	41	12	1		88	0.77
*S—42—1 × S—49—5	1 × 1	49	42	20	1		112	0.76
*S—42—1 × S—60—12	1 × 1	33	41	18	1		93	0.86
*S—42—1 × S—61—8	1 × 1	35	31[1]	6	3		75	0.69
S—60—12 × S—61—8	1 × 1	10	16	3	2		31	0.90
S—61—8 × S—60—12	1 × 1	21	22	6			49	0.69
*D—28—17 × D—28—18	1 × 1	7	8	3	1		19	0.89
*D—28—17 × D—36—12	1 × 1	2	14	8			24	1.25
D—36—12 × D—56—2	1 × 1	15	20	5[2]			40	0.75
S—54—16 × S—49—5	1 × 1	122	107	41	5[3]	1	276	0.75
E—5—2 × E—6—8	1 × 1	89	110[1]	45	3		247	0.85
E—6—8 × E—5—2	1 × 1	60	50	15			125	0.64
Total	1 × 1	636	665	260	29	1	1591	

* Male-sterile plants.

[1] One plant with one acc. iso-chr.

[2] » » » » » » +one st. acc. chr.

[3] » » » » » » +two » » »

frequencies of the different types of gametes their frequencies were calculated for some of the progenies in Table 1. In this connection it was presumed that there were no essential differences in the transmission of the accessory chromosomes between the male and the female side. From the data given in Table 5 it is seen that no such differences have been found. If it is assumed that the relative frequencies of gametes without, with one, and with two accessories are, a, b, and c, respectively, the relative frequencies of the different chromosome numbers among the zygotes obtained with free combination of the gametes would be: without acc. chr. $=a^2$, with one acc. chr. $=2\,ab$, with two acc. chr. $=2\,ac+b^2$, with 3 acc. chr. $=2\,bc$ and with 4 acc. chr. $=c^2$. From the distribution of chromosome numbers among the

different progenies the relative frequencies of the zygotes were obtained and the different types of gametes were calculated. From the above equations it is seen that there are several possibilities for calculating the frequency of gametes. In my calculations, however, the gametes without accessories have been calculated from a^2 and the gametes with one accessory chromosome from $2\,ab$. From the values of a and b the frequency of c has thus been obtained by subtracting $a+b$ from *1*. These estimations may be a little rough but they are considered accurate enough for the purpose of these calculations.

The frequency of gametes with 2 accessories shows a certain variation. Thus, for the last three crosses in Table 1 the frequency of gametes with 2 accessories is abt. 4.4, 3.2 and 1.8 per cent, respectively. If calculating with the sum of progenies in Table 1 — which here, however, is not quite correct as there is some heterogeneity in the material — it seems as though on an average abt. 4.3 per cent of gametes with 2 accessories are produced. The production of such gametes resulted in a certain frequency of plants with 3 and 4 accessories, a small increase of plants with 2 and with 0 accessories, and at the same time a decrease of plants with 1 accessory chromosome. An example of the relation between the different types of gametes produced is given in Table 2. The frequency of the different types of gametes actually obtained is compared in Table 2 with the frequency expected, if it is assumed that only gametes with one and without accessories are produced with random distribution and if at the same time consideration is paid to the elimination of the accessories, which in this cross (S — 54 — 16) × (S — 49 — 5), (*cf.* Table 1), is 20.4 per cent.

The production of gametes with 2 accessories is probably caused by some process of non-disjunction, which may take place during se-

TABLE 2. *Comparison of two methods of calculating gamete frequencies of the cross (S—54—16)×(S—49—5) of the type (20+1 acc. chr.)×(20+1 acc. chr.) (cf. Table 1).*

	The number of accessories in the gametes		
	0	1	2
The frequencies of gametes — calculated on the distribution of the chr. numbers in the F_1-plants	0.6650	0.2915	0.0435
Gamete frequencies without non-disjunction with random distribution, and with 24.64 per cent elimination	0.6232	0.3768	—

veral different developmental stages of the plant. Most probably it is a premeiotic process. It has been established that there is a small variation in number of the accessories in the root-tips within a plant (*cf.* FRÖST, 1956). This fact, however, does not exclude that non-disjunction may take place during the meiotic divisions as well as during the post-meiotic stages. In any case this weak process cannot be compared with the directed mechanism of non-disjunction of the accessories, which generally takes place during the first pollen mitoses in the grasses.

As is further seen from the data in Table 1 there is a considerable variation between different crosses in the degree of elimination of the accessories. In order to obtain an expression for this variation a test of heterogeneity has been made. As the number of plant with 3 and 4 accessories is small these plants have been included in the groups of plants with 2 accessories. In the same way the plants with accessory iso-chromosomes have been included in the respective groups. The iso-chromosome has been counted as 1 acc. chr.

The χ^2-value obtained (54.6860) gave — with 36 degrees of freedom — by extrapolation a P-value between 0.05 and 0.02, and this showed that there was a certain heterogeneity in the material. (The material was also tested with an analysis of variance which gave a quotient of 4.50 and a P-value between 0.01 and 0.001. The analysis of variance is, however, in this case less appropriate as the distribution of values is rather skew.) It is, however, possible that the degree of heterogeneity in the material was slightly decreased since the plants with 3 accessories were included in the groups of plants with 2 accessories.

It is, however, impossible to decide what caused these differences between different crosses. They may be caused by variations of the environmental conditions or by the genotypical constitution of the plants as well as by small differences in the structure and physiology of the accessories. The plants in these crosses were collected in different geographical regions and it must be assumed that their genotypical constitution was extremely variable. The environmental conditions were also very different as the crossings were carried out during different years, in the field and in the greenhouse.

In the reciprocal crosses (E—5—2)×(E—6—8) and (E—6—8)× ×(E—5—2), where the number of plants is high, a considerable difference in the loss of accessories between the two reciprocal cross directions was obtained. The χ^2-value (6.0725) gave with 2 degrees of freedom a P-value between 0.05 and 0.02 (the three plants with 3 acc. chr. have been included in the group of plants with 2 acc. chr.). Unfortuna-

tely, it is impossible to decide whether the greatest elimination has occurred on the male or female side. These differences may be caused by a pre-meiotic non-disjunction which has given rise to a partially chimerical plant: a number of male or female sex cells in one of the parental plants may thus have lacked accessory chromosomes. In the reciprocal crosses (3 b—5) × (8 b—18) and (8 b—18) × (3 b—5) a similar process may have caused some male or female gametes to have 2 accessory chromosomes. (χ^2=9.3921; 3 degrees of freedom; 0.05>P>>0.02. Naturally, the reason for these differences may also be a different degree of meiotic elimination of the accessories between the male and the female side in one of the parental plants. Such a reason, however, is less probable in the crosses (3 b—5) × (8 b—18) and (8 b—18) × (3 b—5) where the number of accessories in one direction is increased above the mean value of the parental plants.

2. Crosses of the type (20+2 st. acc.chr.)×(20+2 st. acc.chr.)

As is seen from the data given in Table 3, eleven crosses of this type have been analysed. If the pairing behaviour of the accessories had been quite regular the daughter plants should have had the same number of accessories as the parental plants. As the accessories seem always to be univalent during MI (FRÖST, 1956) one should, however, expect a more irregular segregation of the accessories at the reduction divisions, as is also evident from the distribution of chromosome numbers among the different progenies in Table 3.

In order to have an idea of the distribution of the accessories at the reduction divisions it was assumed that the accessories segregate at random. If so, a plant with 2 accessories should produce the following types and frequencies of gametes: without, with one, and with two accessories in the ratio 1: 2: 1. It has, however, been shown (Table 1) and is further demonstrated from Table 2 that the accessories are eliminated to a considerable degree during the meiotic divisions. Taking this elimination into consideration, which for the sum of the daughter plants of the eleven crosses in Table 2 is 7.41 per cent, the following types and frequencies should be expected, without, with one, and with two accessories in the ratio 0.2884 : 0.4972 : 0.2143. Using these gametic frequencies, the distribution of chromosome numbers among the zygotes was calculated (*cf.* Table 3). This hypothetical distribution of chromosome numbers was compared with the actual distribution in Table 3. The sum of daughter plants can be used for the comparison since the

TABLE 3. *Number of accessory chromosomes resulting from crosses of the type (20+2 acc. chr.)×(20+2 acc. chr.).*

Parents		F_1							
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1	2	3	4	5	Number of plants	$\bar{x}$ acc. chr. per plant
5a—25 × 8b—31	2 × 2	7	33	40	24	9		113	1.96
8b—31 × 5a—25	2 × 2	10	30	21	19	6		86	1.78
5a—25 × 5a—13	2 × 2	12	16	20	10[1]	2		60	1.57
5a—13 × 5a—25	2 × 2	2	6	11	7	5	1	32	2.31
5a—24 × 8b—23	2 × 2	12	30	48	21[1]	5	2	118	1.86
8b—23 × 5a—24	2 × 2	11	38	41	23	8	1	122	1.85
*D—14—4 × S—60—4	2 × 2	12	23	23[2]	16	5	1	80	1.78
D—61—19 × D—56—5	2 × 2	8	8	20	7	2	2	47	1.85
D—56—5 × D—61—19	2 × 2	8	22	27	16	9		82	1.95
D—61—12 × D—61—19	2 × 2	12	16	30	13	5		76	1.78
D—61—19 × D—61—12	2 × 2	3	6	10	8[1]	—		27	1.85
Total	2 × 2	97	228	291	164	56	7	843	

	Number of acc. chr.				
	0	1	2	3	4
Distribution of chromosome numbers assuming random distribution and 7.41 per cent elimination	70.1376	241.7724	312.5844	179.5590	38.6937

* Male-sterile plants.

[1] One plant with one acc. iso-chr.+two st. acc. chr.

[2] » » » » » » +one » » »

material is homogeneous. The χ^2-value ($\chi^2 = 29.1798$; 4 degrees of freedom; $P < 0.001$) shows a significant difference between the assumed and the actual distributions. The deviation is, however, small and consists of a small excess of plants without, with 4, a few with 5 acc. chromosomes and a deficiency of plants with 1, 2 and 3 acc. chromosomes which results from a small excess of gametes without, with 2, a few with 3 acc. chromosomes and a deficiency of gametes with 1 accessory chromosome.

The frequencies of the different types of gametes suggest that the occurrence of bivalents at MI must be very rare (*cf.* FRÖST, 1956) If a certain fraction of the accessories had formed bivalents and these

had separated regularly at AI, one should instead of a deficiency expect an excess of gametes with 1 accessory chromosome.

The excess of gametes with 2 and without accessories might be explained by the supposition that a small number of accessories had undergone some kind of non-disjunction. The occurrence of this phenomenon was demonstrated when the data from crosses of the type (20+1st acc. chr.)×(20+1st. acc. chr.) were discussed. If such a process is as frequent in plants with 2 accessories, as in plants with 1 accessory chromosome and the accessories undergo non-disjunction independently of each other, then it should be expected that the total frequency of non-disjunction should be about twice as high in plants with two accessories as in plants with only one. It was not possible, however, to calculate to what degree this non-disjunction process might influence the proportion of the different types of gametes. As was pointed out earlier, it was not possible to decide when this process had taken place. Undoubtedly, this process will raise the relative number of gametes with two and without accessories as well as give rise to gametes with 3 accessories.

Another possible cause of the supposed deviation from a random segregation of the univalent accessories might be a tendency of the accessories to move towards the same pole during some of the meiotic divisions and thus in a plant with 2 accessories cause a small excess of two-to-nought distribution. Such a segregation at AI seems to be very frequent for the univalent accessories in *Parthenium argentatum* (CATCHESIDE, 1950).

3. A comparison of the degree of elimination of the accessories in plants with 1 acc.chr. and plants with 2 acc.chr.

As appeared in the data the accessory chromosomes were subjected to a loss from one generation to another. The degree of this loss is, however, relatively low if it is considered that the univalent accessory chromosomes generally divide during first anaphase. From the results there also appeared that there were differences in the degree of elimination between plants with 1 accessory chromosome and plants with 2 accessories. To investigate whether these differences are significant, the percentage of eliminated accessories was calculated for each cross of the types (20+1st. acc. chr.)×(20+1st. acc. chr.) and (20+2st. acc. chr.)×(20+2st. acc. chr.) as well as for the reciprocal crosses of the types (20+1st. acc. chr.)×20 and (20+2st. acc. chr.)×20 (*cf.* Table 5). The mean values obtained are summarized in Table 4.

TABLE 4. *Comparison of crosses involving different numbers of accessories in their elimination of accessories.*

Type of crosses	Number of crosses	Average degree of elimination of the acc. chr. in per cent
(20 + 1 acc. chr.) × (20 + 1 acc. chr.)	19	— 15.6465
(20 + 1 acc. chr.) × 20 and reciprocally	3	— 32.5023
Total	21	— 17.9450 ± 3.583
(20 + 2 acc. chr.) × (20 + 2 acc. chr.)	11	— 6.6917
(20 + 2 acc. chr.) × 0 and reciprocally	3	+ 3.4098
Total	14	— 4.5271 ± 2.582

$t = 3.0385$ $0.01 > P > 0.001$.

The mean value for the meiotic elimination in plants with 1 accessory chromosome (17.9 per cent) agrees very well with the values obtained for plants with 1 accessory chromosome in *Festuca pratensis* (BOSEMARK, 1954). The mean value for plants with 2 accessories (4.5 per cent) differed significantly from the value obtained for plants with 1 accessory chromosome. This value must be assumed as very low when the meiotic behaviour of the accessories is taken into consideration. In *Festuca pratensis*, *Secale cereale* and other species with 2 accessories the rule is a decrease of the meiotic elimination. However, in these species the decrease is caused by the regular formation of bivalents at MI which causes the accessories to segregate regularly and pass undivided to the pole at AI. In *C. scabiosa* it is problematic why the accessories would be eliminated to a lower degree in plants with two accessories than in plants with only one. A simple explanation is that the plants with 1 accessory chromosome might have had a genotypical constitution or might have been subjected to environmental conditions which on the average were more favourable for the elimination of the accessories. Such an interpretation is, however, very improbable. Practically all the plants from the two groups (with 1 accessory and 2, respectively) were from material collected in the same geographical region (southern Sweden and Denmark) and sometimes even from the same population. Naturally, it must be assumed that the genetic variation is large within the material but probably this variation is of the same kind in both categories of plants. It was also impossible to detect any average differences in environmental conditions between the two categories of plants.

Another reason for the differences might be that the accessories in some way influence the behaviour of each other. In this connection it can be pointed out that there was often a small frequency of the accessories which seemed to be associated with each other at diakinesis (cf. FRÖST, 1956). It was impossible by cytological means to detect if and how such false bivalents in *Centaurea scabiosa* would influence the behaviour of the accessories during later stages of meiosis and therefore possibly decrease the meiotic elimination.

4. Crosses of the types (20+1 st. acc.chr.)×20, 20×(20+1 st. acc.chr.) (20+2 st. acc.chr.)×20, 20×(20+2 st. acc.chr.), (20+3 st. acc.chr.) ×20 .....

In several crosses of these types, male sterile mother-plants were used. When both parental plants are male-fertile the degree of self-fertility was found to be very low.

TABLE 5. *Number of accessory chromosomes resulting from crosses of the types 20×(20+1 acc. chr.), (20+1 acc. chr.)×20, (20+2 acc. chr.)×20, 20×(20+2 acc. chr.), (20+3 acc. chr.)×20 ...*

Parents		F_1									Number of plants	$\bar{x}$ acc. chr. per plant
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1	2	3	4	5	6	7	8		
11—2 × 7—3	1 × 0	23	6								29	0.21
7—3 × 11—2	0 × 1	44	20	5							69	0.43
*D—58—15 × D—59—2	0 × 1	58	29	2							89	0.37
7—21 × 8—15	2 × 0	13	24	14	2						53	1.09
8—15 × 7—21	0 × 2	12	15	13	1						41	1.07
11—4 × 7—1	2 × 0	15	19	12							46	0.94
2—2 × 7—9	3 × 0	11	27	19	9	1					67	1.43
*D—9—12 × 8b—12	0 × 3	72	62	48	29	4	1				216	1.23
*D—28—12 × S—57—8	3 × 0	32	25	34	13	—	1				105	1.30
*D—8—19 × S—57—4	0 × 4	17	9	31	20	7	1				85	1.93
*D—8—15 × S—54—19	0 × 4	7	10	11	11	3		1			43	1.93
*D—8—18 × S—50—17	0 × 6	15	13	25	46	32	9	1			141	2.70
*D—8—9 × S—50—5	0 × 7	12	13	27	37[1]	29	12[2]	7	1	1	139	2.96
*D—8—1 × S—50—12	0 × 8	11	7	36	39[1]	58	55[2]	23	6	1[3]	236	3.78

* Male-sterile plants.

[1] One plant with one acc. iso-chr.+2 st. acc. chr.

[2] Three plants with one acc. iso-chr.+4 st. acc. chr.

[3] One plant with one acc. iso-chr.+7 st. acc. chr.

In these crosses it was interesting to study whether there were differences between the male and the female side in the transmission of the accessories, since such differences have been observed in several grass-species e. g., *Anthoxanthum aristatum*, *Zea mays*, *Festuca pratensis* and *Phleum phleoides*.

As can be seen in Table 5 it was impossible to detect any differences between the male and the female side in *C. scabiosa*. Both the meiotic elimination as well as the low frequency of non-disjunction as reported earlier were obviously found on both the male and the female side in about the same proportion, no differences being established in the distribution of the chromosome numbers among the daughter plants in the various cross-combinations. In the reciprocal cross (11—2)× ×(7—3), however, there seems to be a considerable difference in the chromosome distribution as well as in the mean value of the accessories between the daughter-plants. The differences are, however, insignificant ($\chi^2=3.3524$; 3 degrees of freedom; $0.50>P>0.30$).

Two cases have earlier been discussed (p. 407) in which considerable differences in the transmission of the accessories between the male and female side were observed. These cases were, however, exceptions and for *C. scabiosa* it seems justifiable to conclude that there are no differences in the inheritance of the accessories between the male and the female side.

5. Crosses between plants with higher numbers of accessory chromosomes

Crosses between plants with higher numbers are given in Tables 5 and 6. The data show that the decrease of average number of accessories in the progenies when compared to the mother-plants in these crosses is of about the same magnitude as in the crosses between plants with a low number of accessories. Also, there is a variation in the degree of elimination between the different crosses. In the crosses of the types 20×(20+8st. acc. chr.) and 20×(20+6st. acc. chr.) the decrease of accessories is rather small — 6 per cent and 10 per cent respectively. In the cross 20×(20+7st. acc. chr.) and the reciprocal crosses (20+7st. acc. chr.)×(20+7st. acc. chr.) of Table 6, on the other hand, the elimination is more pronounced. The data from the transmission of the accessory chromosome in these crosses do not indicate any higher degree of loss than for crosses involving low numbers.

TABLE 6. *Number of accessory chromosomes resulting from the cross (20+7 acc. chr.)×(20+7 acc. chr.).*

Parents		F_1													
Plant numbers	Number of acc. chr.	Number of acc. chr, 0	1	2	3	4	5	6	7	8	9	10	11	Number of plants	$\bar{x}$ acc. chr. per plant
S—50—5 × S—50—6	7 × 7	—	—	4	5	5	26[1]	12[2]	17	17	3[3]	1	1	91	6.02
S—50—6 × S—50—5	7 × 7	3	3	5	5	14	26[1]	34[2]	28	12	5	5	1	141	5.79

[1] One plant with one acc. iso-chr.+4 st. acc. chr.
[2] » » » » » » +5 » » »
[3] » » » » » » +8 » » »

TABLE 7. *Chromosome numbers in the offspring of four intercrossed plants.*

Plant numbers	Parents	F_1																		Number of plants
	Number of acc. chr.	Number of acc. chr. (st. type and iso-chr.)* 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
5—1, 6—1 6—2, 5—2	± 14 st, 14 st + 1 iso, ± 15 st + 1 iso, 16 st	1	3	5	2	6	17	12	12	12	11	7	8	7	—	1	1	—	1	106

* There are many plants with iso-chromosomes among the F_1-plants as two of the parents have iso-chromosomes. Plants with iso-chromosomes have not been separated here from those with only st. accessories. The plant with 20 accessories had 18 st. acc. chr.+2 acc. iso-chr., whereas the plant with 22 accessories had only st. accessories.

It is probable that still higher numbers of accessories than were tested in these crosses might increase the chance of elimination of the accessories, since high numbers cause a decrease of fertility and general vigour of the plant. The decrease of fertility probably depends in part upon cytological disturbances during meiosis. No controlled crosses between plants with very high numbers of accessories have, however, been made. In Table 7, data have, however, been given from 4 plants with higher chromosome numbers; these were grown in an isolated group and were thus able to flower openly together. The data from the different progenies have been summarized since the determination of the chromosome numbers of the mother plants was uncertain. This isolation group showed that it was only possible to produce plants with up to 20—22 accessory chromosomes. These plants could be maintained in culture without any great difficulties and it is quite possible that plants with still higher numbers of accessories could be produced. However, as in *Festuca pratensis* (BOSEMARK, 1956), plants with high numbers are seldom found in natural populations of *C. scabiosa.*

III. THE INHERITANCE OF THE ACCESSORY ISO-CHROMOSOME

In several progenies resulting from crosses between plants with the standard accessory chromosome some plants had accessory chromosomes of a deviating type. This chromosome is about double the size of the standard type and has a median constriction (FRÖST, 1956), and is very probably an iso-chromosome derived from mis-division of the standard type. The production of plants with the iso-chromosome is more frequent in progenies where the number of the standard accessories in the mother plants is higher (*cf.* Tables 1, 3, 5, and 7). The accessory iso-chromosome is also found in natural populations of *C. scabiosa* and is especially frequent when the standard accessories are numerous.

The transmission of the accessory iso-chromosome was partly studied in plants derived from natural populations and partly in progenies of plants with the standard accessories. Two crosses of the type $20\times \times(20+1$ *acc. iso-chr.*) have been made. In both cases the mother-plants have been male sterile. The data from these crosses are given in Table 8. The result shows that the accessory iso-chromosome has a surprisingly good ability to be transmitted from one generation to the next. The absence of elimination is noteworthy since crosses of the

TABLE 8. *Number of accessory chromosomes resulting from crosses of the type 20×(20+1 acc. iso-chr.).*

Parents		F_1				Number of plants	$\bar{x}$ acc. chr. per plant
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1i	2i	2 st. acc.		
*D/8—1 × K/46b—106	0 × 1i	23	25	—	1	49	0.55
*D/28—14 × K/46b—84	0 × 1i	60	55	1		116	0.49

* Male-sterile plants.

same type with the standard accessory chromosome demonstrated that it was eliminated to a considerable degree (cf. Tables 1 and 4). The ability of the accessory iso-chromosome to be transmitted better than the standard type is further shown in the cross of the type *20×(20+1st. acc. chr.+1 acc. iso-chr.)* (Table 9). In Table 9 B, where the two types of chromosome are compared, there is a considerable difference in the degree of elimination between the standard and the iso-chromosomes. A χ^2-test showed that the difference is highly significant ($\chi^2=8.000$ with a P-value between 0.001 and 0.01). The crosses examined are, however, too few to allow any conclusions to be drawn about any possible differences in their mechanism of inheritance. The ability of the accessory iso-chromosomes to be maintained from one generation to another in these three crosses, is, however, probably caused by a

TABLE 9. *Number of accessory chromosomes in the cross 20×(20+1st. acc. chr.+1 acc. iso-chr.).*

A. Observed chromosome numbers.

Parents		F_1				Number of plants	$\bar{x}$ acc. chr. per plant
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1iso	1st	1st + 1iso		
*D/8—9 × 2b—13	0×(1st.×1iso)	39	25	6	23	93	0.83

* Male-sterile plants.

B. Separate analysis of the st. acc. chromosome and the acc. iso-chromosome.

	Number of acc. chr:s 0	1	Number of plants	$\bar{x}$ acc. chr. per plant
Standard accessory chromosomes	64	29	93	0.31
Accessory iso-chromosomes	45	48	93	0.52

TABLE 10. *Number of accessory chromosomes resulting from the cross 20×(20+1 st. acc. chr.+1 acc. iso-chr.) and reciprocally.*

A. Observed chromosome numbers.

Parents		F_1								Number of plants	$\bar{x}$ acc. chr. per plant
Plant numbers	Number of acc. chr.	Number of acc. chr.									
		0	1st	1iso	2st	1st + 1iso	3st	2st + 1iso	2iso		
8—11 × 7—5	(2st + 1iso) × 0	8	10	5	5	7	2	5	1	43	1.44
7—5 × 8—11	0 × (2st + 1iso)	7	9	8	4	4	2	6	—	40	1.43

B. Separate analysis of the st. acc. chromosome and the acc. iso-chromosome.

		Number of st. acc. chr.				Number of plants	$\bar{x}$ acc. chr. per plant
		0	1	2	3		
The standard accessory chromosome	(2st + 1iso) × 0	14	17	10	2	43	1.00
	0 × (2st + 1iso)	15	13	10	2	40	0.98
		Number of iso-chr.					
		0		1	2		
The accessory iso-chromosome	(2st + 1iso) × 0	25		17	1	43	0.44
	0 × (2st + 1iso)	22		18	—	40	0.45

very low degree of meiotic elimination and not by a mechanism for numerical increase. The distribution of the chromosome numbers among the daughter plants demonstrates that the same mechanism as in the grasses is not effective.

Two chromosomes of the same morphological type as the standard accessory chromosomes appeared in one of the daughter plants from the cross (D 18—1)×(K/46 b—106) (Table 8). The new type may have been produced through mis-division of the accessory iso-chromosome. However, it is also possible that the plant was the result of an accidental cross.

One reciprocal cross of the type *20*×(*20*+*2st. acc. chr.*+*1 acc. iso-chr.*) has been made. The data are given in Table 10.

From the mean values of the accessories for the two progenies it is clear that there has been some elimination of the accessories but that this loss has been slight. To get a better survey of the distribution of the chromosome numbers among the daughter plants the two types of accessories have been compared separately (Table 10 B). Thus there was no difference in inheritance between the male and female side for either of the two types of accessories since the distribution of chromosome numbers as well as the mean values of accessories were about the same.

In conclusion, the accessory iso-chromosomes have at least the same ability as the standard accessories to maintain themselves from one generation to the next. As for the standard type it has not been possible to demonstrate any differences in the transmission of the iso-chromosome between the male and female side.

IV. CONCLUSIONS AND DISCUSSION

The inheritance of the accessory chromosomes in *C. scabiosa* has been studied in about 50 cross combinations. Most of these crosses have been done in order to study the accessory chromosome of the standard type. In the following discussion only the results obtained from crosses with the standard accessory chromosome are taken into consideration. This type was also the most frequent one in natural populations of *C. scabiosa*. It can, however, be pointed out that no essential differences in the inheritance between the accessory iso-chromosome and the standard type have been proved.

The data showed no essential differences in the inheritance of the accessories between the male and the female side. The accessories are,

however, not transmitted without disturbances since some are generally eliminated from one generation to another. The average degree of the meiotic elimination in *C. scabiosa* is, however, surprisingly low considering that the accessories always seem to be univalent at MI and often divide at AI. The results obtained from crosses of the type (20+1 acc. chr.)×(20+1 acc. chr.) showed that there was a certain variation between different plants in the degree of elimination. Furthermore, there seemed to be an average difference as to the degree of the elimination between plants with one and those with two accessories. The reason for this difference could not be satisfactorily explained.

As a result of the way in which the accessory chromosomes were distributed to the gametes, plants with a higher number of accessories than in the parental plants, were obtained, at least in some cross combinations. This nearly random distribution of the univalent accessories during the meiotic divisions in *C. scabiosa* cannot, however, be considered as a mechanism for numerical increase since there is generally higher number of plants with lower mean numbers of accessories than in the parental plants. Non-disjunction of the accessories that sometimes occurs produces plants with a number of accessories higher than expected only from random distribution of the parental number of accessories. This process is, however, rather rare and it cannot be compared with the directed non-disjunction process during the first or second pollen mitosis which is found in several grass-species, e. g. *Secale cereale* (MÜNTZING, 1945, 1946), *Zea mays* (RANDOLPH, 1941, ROMAN, 1947), *Anthoxanthum aristatum* (ÖSTERGREN, 1947). *Festuca pratensis* (BOSEMARK, 1950, 1954) and *Phleum phleoides* (BOSEMARK, 1956). In *Secale cereale* there is also a directed non-disjunction of the accessories at the first mitosis in the embryo-sac (HÅKANSSON, 1948). Because of this mechanism, the grass-species mentioned have a very good ability to maintain or even increase their number of accessories from one generation to another in spite of a considerable meiotic elimination. ÖSTERGREN (1947) considers that the accessory chromosomes in these species may maintain themselves in the population mainly because of this ability to increase their number from one generation to another. This conclusion is drawn in relation to the earliest detectable genetic effect which gave evidence of a negative effect on general vigour and fertility, at least, when the number of accessories is high. ÖSTERGREN considers, therefore, that the accessories may exist as parasites and thus mainly fight for their own existence. This theory which is based on the occurrence of a well-developed mechanism for numerical in-

crease can scarcely be accepted for the accessory chromosomes in *C. scabiosa*, where it is more likely that the loss of accessories, at least under some conditions, is compensated by natural selection for plants having accessory chromosomes. If this was not the case, it is difficult to understand how they can maintain themselves in natural populations. Studies of the geographical distribution of plants with accessories have shown that especially in some geographical areas accessory chromosomes are very common (data given by FRÖST in MÜNTZING, 1954). Indication of a stimulating effect on the vegetative development of the accessories in *C. scabiosa* has also been obtained (FRÖST, 1954).

No evidence of any mechanism for numerical increase of the accessories in accordance with results in *C. scabiosa* was obtained in *Caltha palustris* (REESE, 1954). The same may also be true of the accessories in *Poa alpina* (MÜNTZING, 1954, HÅKANSSON, 1954).

SUMMARY

(1) The inheritance of the two types of accessory chromosomes in *C. scabiosa*, the standard type and the accessory iso-chromosome, was studied.

(2) There was no evidence of an effective mechanism for numerical increase of the accessories. Instead, it was found that there is, in general, a small loss of accessories from one generation to the next. The accessories also seem to be transmitted in the same manner on the male and the female side.

(3) When calculating the expected frequencies of gametes in plants with two accessories, the distribution of the accessories at the meiotic divisions did not seem, in spite of their univalent character, to be at random. The small deviation found was assumed to depend upon the small percentage of non-disjunction of the accessories, which produced plants with a higher number of accessories than expected.

(4) When comparing the average percentage of eliminated chromosomes between plants with only one and those with two accessories, it was found that the loss of accessories was higher in plants with one accessory chromosome than in plants with two. This difference was discussed in relation to the fact that the accessories in *Centaurea scabiosa* always seemed to be univalent at MI (*cf.* FRÖST, 1956).

(5) No difference was obtained in the mode of inheritance between accessory iso-chromosomes and accessory chromosomes of the standard type.

(6) Since there was usually a small loss of accessories from one generation to the next and as plants with accessories in some geographical regions are very common, it is assumed that the loss of accessories, at least under certain conditions, is compensated for by natural selection of plants with accessory chromosomes.

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AB0 BLOOD GROUPS OF NORTHERN AND CENTRAL SWEDEN

By *LARS BECKMAN*

THE STATE INSTITUTE FOR HUMAN GENETICS, UPPSALA, SWEDEN

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I. INTRODUCTION

1. Some pertinent historical data

THE investigation area, which is located north of the Mälar District, is, as a cultural and historical province, widely different from southwest Sweden (ARBMAN 1947).

The first evidence of human settlement in southwestern Sweden dates from about 9000 B. C. The tribes of this stone culture were hunting and fishing people who immigrated from the southwest, following the receding ice edge. This culture spread northwards up to region IV [1] and further to Norway. In the northernmost part of Scandinavia, there was at the same time a special stone culture presumably with strong eastern connections (the Komsa culture). From Lappland (regions VI and VII), we have a unique series of findings of heavy quartz articles that probably are connected with the North-Scandinavian stone culture. Thus, very early we find two different ethnic culture elements in Scandinavia — one with southwestern and one with eastern elements.

From about 3000 B. C., we find a Megalithic culture in southwest Sweden with strong connections with Denmark and southwest Europe. Later (2500 B. C.), another group of people invaded central and eastern Sweden. This people had a different type of graves (one-man graves), boatshaped axes and a special kind of ceramics. This was probably an Indo-European people who came in along the east coast of the Baltic. From the dominance-area around Lake Mälaren, they spread further north to Norway and along the east coast of Sweden to the Arctic Sea.

[1] When discussing regional problems in this paper, the author has chosen to refer to the figures on the map (Fig. 1). This has been done to avoid misunderstandings because of the often complicated divisions of Sweden into »landskap» and »län». The former division is historical and the latter is administrative. These different units are to some extent overlapping.

In north Sweden there was formed at the same time a stone culture similar to the circumpolar slate culture. Fragments of this culture have been spread throughout the whole Scandinavian peninsula, but not to Denmark. Evidently this slate culture cannot have a south-Scandinavian origin, but must have come from the northeast. Other evidences that show an eastern orientation of north Scandinavia during neolithic times are findings of the Olonetz flint and Finnish slate objects.

The introduction of the Bronze Culture did not give any immigration of new peoples. The leading stratum was developed from the boat-axe people. During the end of the Bronze Age, the *Svears* of the Mälar District, which was the centre for furtrading, became leading and there was lively communication with Norway and Finland. It has been stated that the Lapps, during the period of deteriorating climatic conditions round 500 B. C., moved into the Scandinavian peninsula from the colder places near the Arctic Sea where they had lived earlier.

The first written historical data are given by TACITUS (98 A. D.), who gives a short description of the *Svears*. Later (about 500 A. D.), further data are given by JORDANES and PROKOPIOS. PROKOPIOS describes the Scandinavian peninsula as having 13 groups of people. Among others, he mentions Skrithifinoi (the Lapps).

During the Viking Age, there were lively trade communications from central Sweden eastwards even as far as Constantinople. Vikings from Roslagen in region I were probably identical to the Ruser that ruled some of the Russian kingdoms from the ninth century (e. g. NOWGOROD). During 1100—1300 A. D., there were many crusades from Sweden to Finland which gave a strong Swedish colonisation of the southern and northwestern parts of Finland (thus, the name Helsingfors from Hälsingland in Sweden). About 1300 A. D., the Swedish settlements in the northern parts of the country did not go north of Umeå, north of this settlement being only nomadic Lapps. The trade with the Lapps was monopolized by Finnish tradesmen, or *birkarlar*, who were organized in at least three societies (Piteå, Luleå and Torneå). The influence of the *birkarlars* persisted as long as until the seventeenth century.

About 1580, large numbers of Finns immigrated to central Sweden, settling mainly in the wooded districts of Värmland, Hälsingland (region III) and Dalecarlia (region II). The immigration proceeded until about 1700 and probably totalled 40.000 immigrants.

The colonisation of the inner parts of regions VI and VII, which was comparatively late (mainly in the beginning of the nineteenth century), has been studied in detail for some districts by BYLUND (1956).

He studied genealogically the origin of the people that colonized the inner parts of northern Sweden. From his maps we notice the interesting fact that there are two main passages of Finnish immigration — one in region VII (the county of Norrbotten) and one in the southern parts of region VI (the county of Västerbotten).

2. Previously known blood group data

Data on the distribution of the AB0 blood groups in Sweden have been summarized by LUNDMAN (1940), and further information was given by MOURANT (1954). These data consist of small samples mostly collected in towns.

The A-frequency is higher than 30 per cent in two areas, namely in the northern part of the country and in the area around Lake Mälaren. The whole province of Lappland is said to have frequencies higher than 35 per cent.

The B-frequency nowhere exceeds 10 per cent. The isogene for 10 per cent of B follows the border between Finland and Sweden. MOURANT claims that the Baltic and the Gulf of Bothnia represent a marked border between a western region with low B-frequencies and an eastern region with high B-frequencies. Lappland is the only area with frequencies lower than 5 per cent.

No part of the country has a higher *0-frequency* than 65 per cent. The northern part of the country has values lower than 60 per cent, and the Lapps have extremely low frequencies.

II. PRESENT INVESTIGATION

1. Material and methods

The AB0 blood groups are registered in the medical examination files at the Swedish Army Hospitals. The same files also give information about the birth parish. The blood typing is made by physicians at the military hospitals (one hospital for each regiment).

The files of individuals born in north and central Sweden were sorted out and grouped. Northern and central Sweden have been defined as shown in Fig. 1. Information for the AB0 blood group distribution and birth places was obtained for 44.766 conscripts. The age of the conscripts varied between 20 and 45 years.

The fact that the data include only males is known to be of no

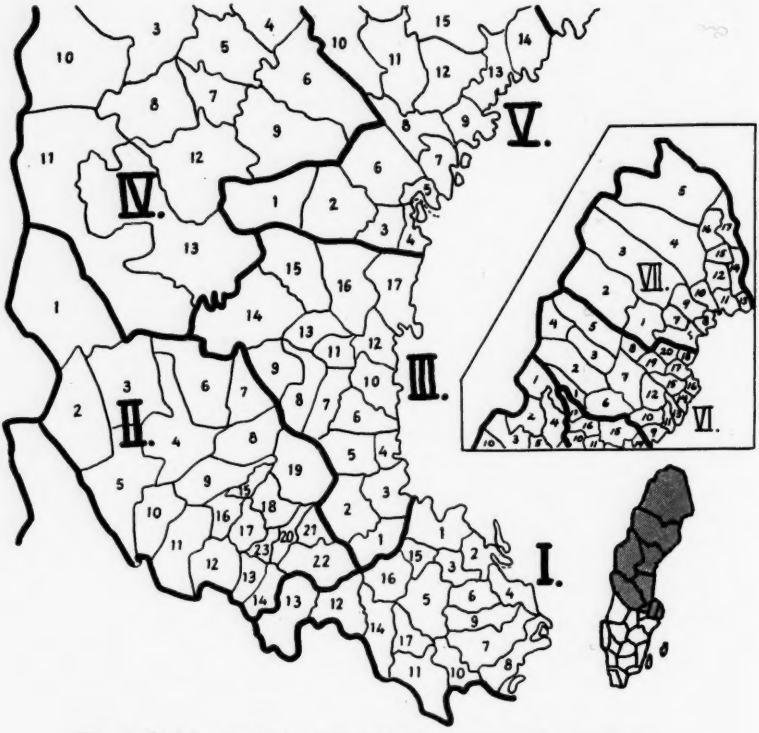

Fig. 1. Division in regions. Investigation area on the small map.

consequence in a study of the geographical distribution of the AB0 blood groups. The internal consistency of the sample may be affected by the fact that the typing has been done by different people which is a common feature of all large samples. This error, however, can be measured and will be discussed below.

An additional sample of 3.769 individuals (males and females) born in different parishes in region IV was collected from the files of the Swedish Red Cross. The typing of these individuals was made in Stockholm by serologists.

2. Subdivision in regions

An analysis of the geographical distribution of the AB0 blood groups has to be based on some kind of arbitrary subdivision. This problem

was discussed thoroughly in a recent paper by FRAZER ROBERTS (1953). He analyzed a large sample from the north of England, starting with very small areas. Subsequently he increased their size by the addition of neighbouring areas and tested every step with regard to heterogeneity within and between the regions. The final result was a subdivision into 52 areas which appeared to give the best description of the geographical variation. The classification of the individuals was by home addresses.

In the present material, the individuals have been classified according to their birth parishes, and the basic unit for the geographical classification was the parish. The classification by birthplace has clearly an advantage as compared to the classification by home address. However, as the parishes are administrative units of varying areas and population, they cannot be compared directly. The areas of the parishes in north Sweden are very large as compared to the size of those of central Sweden, for example.

The original records with the subdivision by parishes have been filed at the State Institute for Human Genetics in Uppsala. As a first step, the material was divided into seven regions according to the administrative subdivision of the counties (Swedish »län»). These regions correspond roughly to the military enrolment areas. Region I, however, includes the counties of Stockholm Uppsala and half the county of Västmanland. These areas were taken together because the counties are comparatively small and represent a very artificial subdivision in comparison with the other regions. These seven regions were divided further into subregions.

Geographical and historical considerations have, in most instances, determined this classification. An example of a subdivision based on geographical factors is that of Härjedalen (southern part of region IV) into a western subregion (subregion IV: 11) which is a mountain area, and an eastern area which is lowland. A number of parishes in Uppland with the names ending in *-hundra* have been assembled to form one of the subregions. This is an example of a subdivision based on historical factors as these names are connected with the old war organization of the *Svears*. The blood group distributions of all subdivisions are given in tables 1—7.

3. Consistency of the data

In tables 1—7 the actual number of tested individuals is given for each blood group. The gene frequencies have been calculated according

TABLE 1. *Blood group distribution of region I (cf. Fig. 1).*

Sub-regions	A	B	AB	0	n	χ^2	p	q	r	p+q+r
1	225	40	16	192	473	0.15	30.0	6.1	63.9	99.79
2	107	42	16	67	232	1.27	31.7	13.5	54.8	98.59
3	98	35	21	91	245	1.81	28.1	12.1	59.8	101.41
4	111	41	10	77	239	3.46	30.0	11.4	58.6	98.00
5	192	33	26	150	401	1.91	32.6	7.7	59.7	99.02
6	119	35	16	105	275	0.03	28.6	9.7	61.7	100.19
7	212	38	23	147	420	0.21	33.6	7.5	58.9	100.32
8	71	13	10	73	167	2.59	28.0	7.1	64.9	101.52
9	137	25	16	92	270	0.26	34.1	7.9	58.0	100.46
10	78	19	6	71	174	0.34	28.2	7.4	64.4	99.40
11	108	26	9	74	217	1.14	32.3	8.4	59.3	98.93
12	144	31	23	116	314	2.62	31.4	8.9	59.7	101.37
13	147	35	11	114	307	1.52	30.5	7.8	61.7	99.04
14	287	55	27	188	557	0.24	34.0	7.6	58.4	99.70
15	161	23	7	107	298	2.78	34.1	5.2	60.7	99.06
16	206	51	18	133	408	2.49	33.0	8.9	58.1	98.79
17	82	21	9	67	179	0.02	29.9	8.8	61.3	99.84
Total	2485	563	264	1864	5176	0.415	31.5	8.4	60.1	99.87

TABLE 2. *Blood group distribution of region II (cf. Fig. 1).*

Sub-regions	A	B	AB	0	n	χ^2	p	q	r	p+q+r
1	108	21	9	98	236	1.06	29.1	5.9	65.0	99.24
2	119	28	2	104	253	3.40	29.0	5.8	65.2	98.70
3	166	42	12	191	411	4.65	25.1	5.9	69.0	98.91
4	283	96	35	312	726	0.13	25.1	9.6	65.3	100.18
5	257	50	23	135	465	1.64	37.1	8.3	54.6	99.03
6	169	60	18	129	376	2.39	29.4	11.0	59.6	98.72
7	63	24	9	68	164	0.01	25.1	10.5	64.3	100.12
8	147	44	12	152	355	0.87	25.8	8.3	65.9	99.38
9	187	43	12	201	443	0.72	25.8	6.4	67.8	99.56
10	162	51	31	135	379	1.88	29.8	11.5	58.7	101.17
11	137	58	22	111	328	0.27	28.3	13.2	58.5	99.50
12	88	26	11	79	204	0.002	28.3	9.5	62.2	100.04
13	145	41	20	138	344	0.37	27.8	9.3	62.9	100.46
14	57	25	7	60	149	0.27	24.6	11.5	63.9	99.38
15	110	19	11	107	247	0.60	28.4	6.3	65.3	100.57
16	101	24	7	110	242	0.26	25.6	6.6	67.8	99.64
17	458	107	44	388	997	0.04	29.6	8.0	62.4	99.92
18	126	14	14	101	255	3.54	32.6	5.6	61.8	101.41
19	183	38	15	142	378	0.15	31.1	7.5	61.4	99.73
20	146	35	7	117	305	0.003	31.8	9.0	59.2	100.05
21	97	21	9	94	221	4.95	29.4	6.9	63.7	101.86
22	138	24	12	138	312	0.54	27.9	6.0	66.1	100.46
23	56	27	13	66	162	1.17	24.1	13.2	62.7	101.32
Total	3503	918	355	3176	7952	1.78	28.3	8.3	63.4	99.80

TABLE 3. *Blood group distribution of region III (cf. Fig. 1).*

Sub-regions	A	B	AB	0	n	χ^2	p	q	r	p+q+r
1	176	25	19	121	341	1.17	34.5	6.6	58.9	100.80
2	223	43	18	179	463	0.16	30.8	6.8	62.4	99.76
3	229	45	19	159	452	0.58	32.9	7.4	59.7	99.49
4	87	16	7	49	159	0.55	36.2	7.6	56.2	99.10
5	165	29	12	126	332	0.23	31.7	6.4	61.9	99.67
6	194	36	10	118	358	0.55	36.4	6.6	57.0	100.56
7	150	45	19	130	344	0.34	29.1	9.8	61.1	100.47
8	136	20	9	97	262	0.13	33.3	5.7	61.0	99.73
9	161	18	6	107	292	1.13	34.7	4.3	61.0	99.32
10	160	25	10	90	285	1.41	36.6	6.4	57.0	99.01
11	107	18	8	64	197	0.42	35.6	6.9	57.5	99.34
12	105	12	9	85	211	1.01	32.1	5.1	62.8	100.78
13	81	29	7	66	183	2.13	28.2	10.4	61.4	98.37
14	108	16	10	107	241	1.23	28.5	5.5	66.0	100.77
15	117	26	14	87	244	0.04	31.9	8.6	59.5	100.20
16	159	31	16	113	319	0.001	32.9	7.6	59.5	99.98
17	148	42	10	130	330	2.88	28.0	8.2	63.8	98.77
Total	2506	476	203	1828	5013	4.025	32.3	7.0	60.7	99.62

TABLE 4. *Blood group distribution of region IV (cf. Fig. 1).*

Sub-regions	A	B	AB	0	n	χ^2	p	q	r	p+q+r
1	153	11	22	100	266	1.92	35.6	2.6	61.8	99.27
2	98	22	7	56	183	2.19	35.0	8.3	56.7	98.29
3	115	43	24	156	378	1.88	27.3	9.2	63.5	100.96
4	202	46	20	170	438	0.01	29.8	7.9	62.3	99.94
5	151	39	12	114	316	2.06	30.7	8.4	60.9	98.85
6	164	19	13	139	335	1.16	31.4	4.9	63.7	100.64
7	145	35	27	124	331	4.17	30.4	9.7	59.9	101.72
8	100	28	13	90	231	0.10	28.6	9.3	62.1	100.30
9	113	17	5	65	200	2.19	36.2	5.7	58.1	98.63
10	96	12	6	91	205	0.09	29.0	4.5	66.5	100.21
11	120	20	7	109	256	0.22	29.0	5.4	65.6	99.68
12	204	32	16	160	412	0.01	31.7	6.0	62.3	100.06
13	200	43	15	156	414	1.12	30.8	7.3	61.9	99.31
Total	1901	367	167	1530	3965	0.151	30.8	7.0	62.2	99.92

TABLE 5. *Blood group distribution of region V (cf. Fig. 1).*

Sub-regions	A	B	AB	0	n	χ^2	p	q	r	p+q+r
1	89	22	4	75	190	2.87	28.8	7.2	64.0	98.46
2	180	32	16	141	369	0.003	31.6	6.7	61.7	100.07
3	120	14	14	92	240	3.31	33.3	5.9	60.8	101.47
4	230	66	37	197	530	1.36	29.4	10.2	60.4	100.78
5	154	39	16	101	310	0.76	32.9	9.4	57.7	99.21
6	127	32	13	142	314	0.12	25.5	7.4	67.1	100.23
7	165	38	24	130	357	1.06	31.2	9.1	59.7	100.82
8	267	74	33	261	635	0.27	27.4	8.8	63.8	100.28
9	163	40	12	180	395	0.23	25.4	6.8	67.8	99.73
10	156	28	14	177	375	1.74	25.9	6.0	68.1	100.72
11	100	35	9	166	310	0.19	19.9	7.4	72.7	100.46
12	135	39	9	166	349	0.86	23.4	7.2	69.4	99.46
13	195	51	17	270	533	0.13	22.4	6.6	71.0	100.16
14	138	39	15	157	349	0.09	25.0	8.1	66.9	100.19
15	167	34	11	247	459	0.14	21.8	5.0	73.2	100.15
16	119	16	6	109	250	0.94	29.0	4.5	66.5	99.40
17	89	19	9	104	221	0.58	25.4	6.5	68.1	100.56
Total	2594	618	259	2715	6186	1.732	26.6	7.3	66.1	100.20

TABLE 6. *Blood group distribution of region VI (cf. Fig. 1).*

Sub-regions	A	B	AB	0	n	χ^2	p	q	r	p+q+r
1	94	26	5	121	246	1.12	22.8	6.5	70.7	99.32
2	216	47	11	222	496	2.03	26.4	6.1	67.5	99.31
3	164	58	14	103	339	7.45	31.5	11.4	57.1	97.45
4	81	15	2	52	150	4.33	33.5	5.9	60.0	97.87
5	131	31	10	107	279	0.91	29.8	7.7	62.5	99.24
6	105	17	6	174	302	0.42	20.5	3.9	75.6	100.27
7	121	46	12	108	287	1.91	26.9	10.7	62.4	98.79
8	72	20	15	57	164	1.70	31.2	11.2	57.6	101.72
9	212	44	9	222	487	18.48	25.1	5.7	69.2	98.04
10	262	75	18	246	601	4.04	27.1	8.1	64.8	98.65
11	496	123	39	403	1061	3.96	29.8	8.0	62.2	99.17
12	236	62	28	186	512	0.02	30.5	9.2	60.3	99.91
13	133	21	9	106	269	0.05	31.3	5.8	62.9	99.84
14	270	82	17	254	623	6.58	26.7	8.3	65.0	98.68
15	202	77	20	219	518	1.42	24.5	9.9	65.6	99.29
16	142	93	22	197	454	0.55	20.1	13.6	66.3	99.51
17	321	115	35	269	740	3.43	28.1	10.7	61.2	98.96
18	162	45	11	118	336	4.35	30.6	8.8	60.6	98.34
19	113	39	17	100	269	0.01	28.1	11.0	60.9	100.10
20	89	18	6	65	178	0.62	31.9	7.0	61.1	99.22
Total	3622	1054	306	3329	8311	27.59	27.5	8.6	63.9	99.23

TABLE 7. *Blood group distribution of region VII and total regions I—VII (cf. Fig. 1).*

Sub-regions	A	B	AB	0	n	χ^2	p	q	r	p+q+r
1	191	46	22	132	391	0.10	32.6	9.1	58.3	99.75
2	98	18	9	44	169	0.86	39.7	8.4	51.9	98.76
3	165	25	10	127	327	0.26	31.9	5.5	62.6	99.67
4	397	74	16	237	724	14.02	34.7	6.5	58.8	98.11
5	290	52	21	178	541	1.89	35.0	7.0	58.0	99.16
6	312	84	30	287	713	0.52	27.9	8.4	63.7	99.64
7	177	54	16	179	426	0.66	26.2	8.6	65.2	99.49
8	185	35	31	185	436	10.19	28.6	7.8	63.6	101.99
9	310	73	32	217	632	0.47	32.4	8.6	59.0	99.59
10	273	38	25	189	525	0.65	34.2	6.2	59.6	100.46
11	327	78	29	351	785	0.00	26.0	7.1	66.9	100.00
12	280	29	11	237	557	0.29	30.9	3.7	65.4	99.79
13	191	59	29	172	451	0.42	28.4	10.3	61.3	100.46
14	240	75	35	203	553	0.06	29.1	10.5	60.4	100.16
15	124	17	7	74	222	0.78	36.1	5.6	58.3	99.23
16	172	10	8	76	266	2.09	42.9	3.4	53.7	101.02
17	240	39	30	136	445	0.77	37.2	8.1	54.7	100.67
Total	3972	806	361	3024	8163	1.958	29.5	7.8	62.7	99.79

Total data (Region I—VII).

	A	B	AB	0	n	χ^2	p	q	r	p+q+r
Total	20583	4802	1915	17466	44766	15.78	29.5	7.8	62.7	99.75

to Bernstein's efficient estimates and are given in the tables as per cent. STEVENS (*cf.* COTTERMAN, 1954) has shown that these estimates are fully efficient, and they also lead to a simple formula for a direct calculation of χ^2 (with one degree of freedom),

$$\chi^2 = 2n\left(1 + \frac{r}{p \cdot q}\right) \cdot D^2;$$

where

$$D = 1 - (p+q+r).$$

The values of χ^2 calculated by this formula are given in tables 1—7 for each region and subregion. In the same tables the sum of the preliminary estimates (p, q, and r) is also given.

The consistency of group proportions judged by the χ^2-test (with

one degree of freedom) is insufficient for the total of the data. Ten of the 124 subregions into which the sample is divided give a significant χ^2-value, which is clearly more than theoretically expected. The errors seem to lie mostly in a deficiency of AB:s, which is a feature common to all large samples (*cf.* FRASER ROBERTS, 1953, DOBSON and IKIN, 1946). This is commonly looked upon as due to misclassification of A_2B:s as B:s. This source of error is probably high in this sample since the typing was not made by expert serologists. Only the frequency of the A gene is affected by this sort of misclassification, and attention has been paid to the inconsistent series in the discussion of the geographical variation.

4. Heterogeneity

The variation in the distribution of the three genes within and between the seven regions has been examined by calculating χ^2 for heterogeneity for the relative numbers of A+AB and B+AB. The heterogeneity of these two ratios gives a reasonable estimate of the variation in the distribution of A and 0 genes and B genes respectively.

The result is shown in table 8. As expected, most of the areas show a pronounced heterogeneity for the distribution of the three genes within the seven main regions. The distribution of the B genes tested by relative numbers of B+AB has a tendency to be more heterogeneous.

TABLE 8. *The heterogeneity within and between the main regions.*

Region	A+AB		B+AB		Degrees of freedom
	χ^2	P	χ^2	P	
I	4.57	$P > 0.99$	46.39	$P < 0.001$	16
II	62.58	$P > 0.001$	87.66	$P < 0.001$	22
III	21.92	$0.27 > P > 0.1$	18.24	$0.5 > P > 0.3$	16
IV	15.01	$0.3 > P > 0.2$	49.60	$P < 0.001$	12
V	79.28	$P < 0.001$	42.21	$P < 0.001$	16
VI	75.82	$P < 0.001$	103.17	$P < 0.001$	19
VII	102.71	$P < 0.001$	79.18	$P < 0.001$	16

S:a I—VII A+AB; $\chi^2 = 155.8$ (6 degrees of freedom) $P > 0.001$
S:a I—VII B+AB; $\chi^2 = 46.9$ (6 degrees of freedom) $P > 0.001$

5. Geographical variation

The geographical distribution of the genes is shown in Figs. 2—5. As can be seen from Fig. 2, there are two main areas of high A-

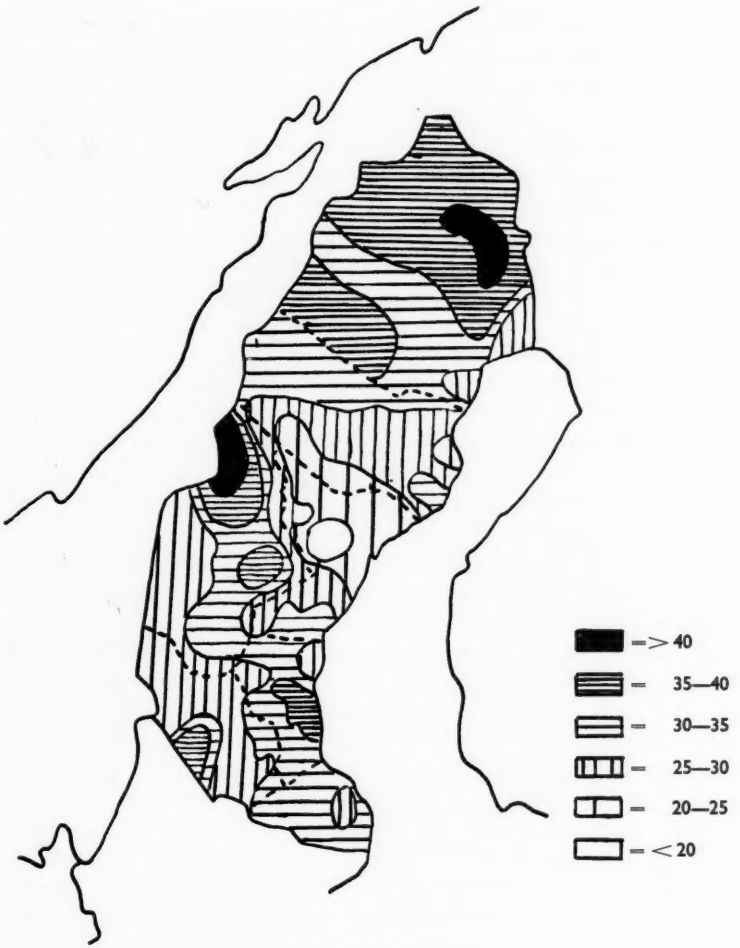

Fig. 2. A-gene frequency.

frequency; one in the northeastern part of the country (region VII) and one in the northern part of region IV. The *A-gene-frequency* shows a wide range of variation with great differences between adjacent regions, e. g. the northern part of subregion IV: 1 has a frequency of about 40 per cent while the adjacent region V has frequencies around 20—25 per cent (sometimes even below 20 per cent).

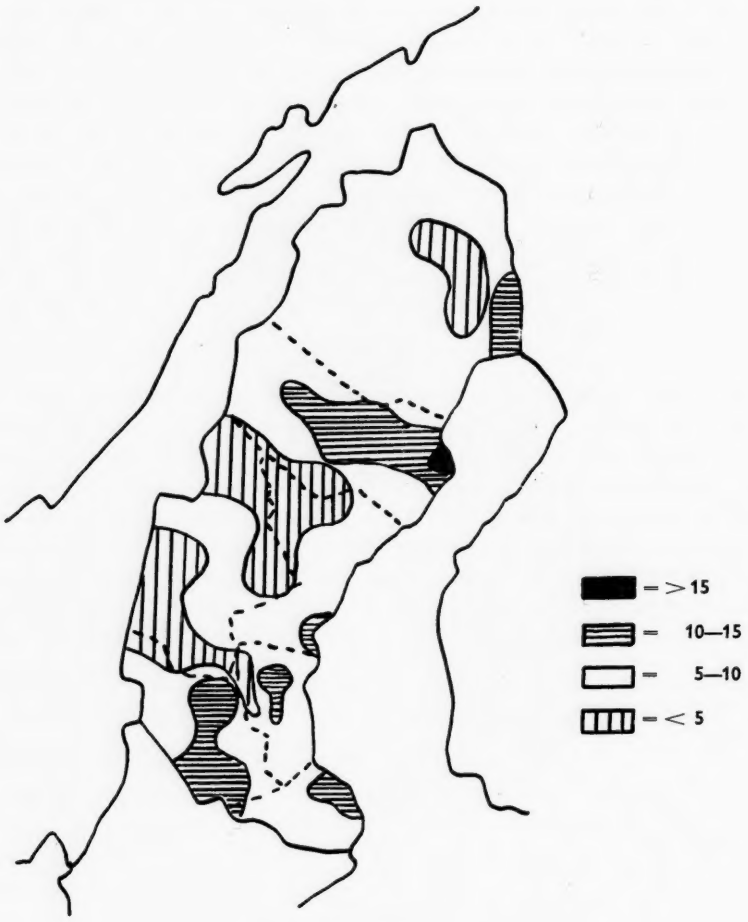

Fig. 3. B-gene frequency.

There are several areas with *B-frequencies* higher than 10 per cent (*cf.* Fig. 3). A rather wide variation ranging from about 2 per cent (subregion IV: 1) to 15 per cent (a part of subregion VI: 16) has been observed. All the main regions except region III were heterogeneous for B. Areas with higher than 10 per cent are mainly located in the eastern part of region I (Roslagen), in region II (Dalecarlia) and in the north-

eastern part of region VI. Minor areas are found in regions V and VII. Frequencies lower than 5 per cent were found along the valley of the river Kalix (in region VII), in the northeastern and southwestern parts of region IV and in the western part of region V.

All Lapps who have been examined so far show low B-frequencies. However, low B-frequencies have been found in areas which have not been inhabited by Lapps in historical times. A hypothesis of an intermixture with Lapps would not account for such low frequencies.

The Baltic and the Gulf of Bothnia do not appear to be such sharp serological borders as has been supposed previously. The higher frequencies were found more towards the west than Finland, i. e. in the eastern parts of Sweden. As also low frequencies were much more common than previously reported or known, the variation in B-gene frequencies was, on the whole, found to be very great as compared with e. g. England and Ireland. The B-decrease westwards in Scandinavia shows a steeper decline than the B-decrease in central Europe.

The most interesting feature is the high *0-frequency* area in region V (*cf.* Fig. 4). To this area belong also the southern parts of region VI. Many adjacent subregions have values higher than 70 per cent. Even values higher than 75 per cent are found. This, in combination with the low A-frequencies, seems to show a very close genetical relationship with Ireland, northwestern England and Iceland. HACKETT et al. (1956) state that there is a closer genetical relationship between Iceland and Ireland-England than between Iceland and Scandinavia, which is said to have widely different frequencies. Our data, however, contradict this opinion which was based on the scanty data on Scandinavia available at that time. Scandinavia has typical west-Atlantic AB0-distributions in some areas, and also shows rather »easterly» distributions with high B-incidences. It might be worth noting that the main area with high 0-frequencies (and low A-frequencies) is found between the two eastern B-introgressions in central and northern Sweden.

6. Rh-data

From the investigation in region IV (the county of Jämtland) made by the Swedish Red Cross, data of D-positives and -negatives have been obtained. Table 9 shows the distribution of AB0 and Rh-blood groups for 23 parishes covering about half the county. The material has been divided into 18 subregions (see Fig. 5). The heterogeneity-χ^2 for the relative number of Rh-negatives gave a χ^2-value of 39.9 for 17

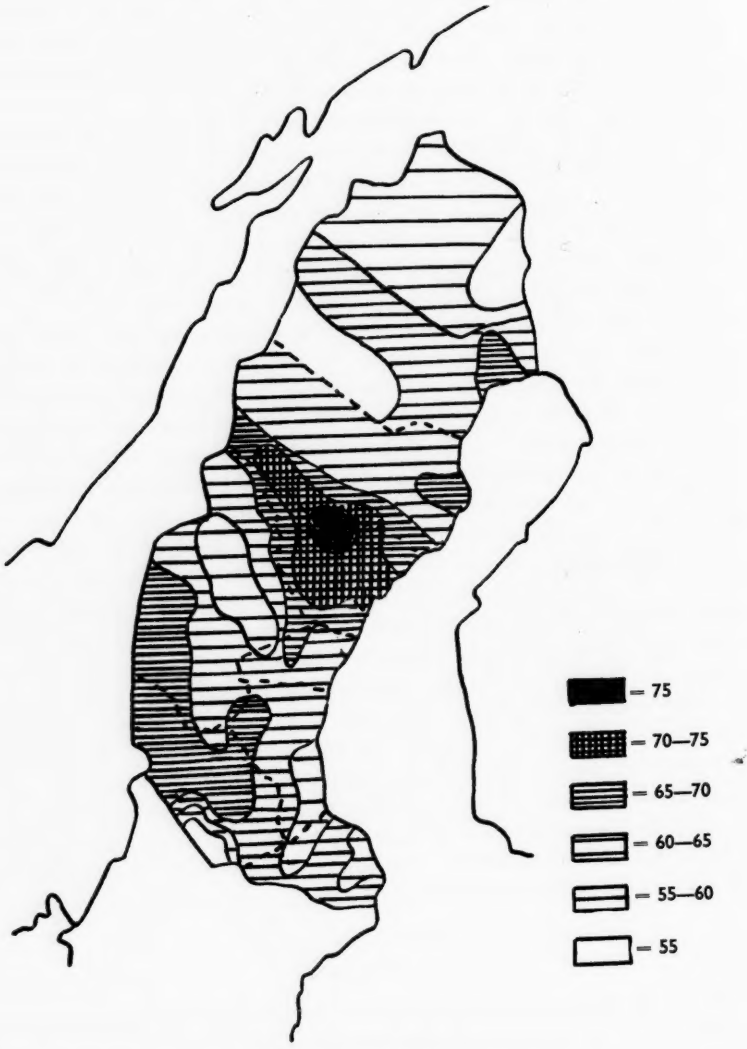

Fig. 4. O-gene frequency.

TABLE 9. *Distribution of AB0- and Rh-bloodgroups in region IV.*

Parishes	A	B	AB	0	N	Rh-neg. %	
Östersund o. Frösön	175	45	16	148	384	15.6	+
	32	7	7	25	71		−
Ås	99	22	11	75	207	17.2	+
	20	5	0	18	43		−
Lit o. Kyrkås	68	26	4	53	151	16.6	+
	8	5	1	16	30		−
Rödön	72	33	3	67	175	12.9	+
	9	4	3	10	26		−
Hammerdal o. Gåxsjö	297	71	36	226	630	14.9	+
	61	12	4	33	110		−
Häggenås	115	27	13	82	237	22.0	+
	27	6	3	31	67		−
Alsen o. Näskott	63	21	8	45	137	19.9	+
	15	5	1	13	34		−
Revsund	70	9	7	51	137	11.6	+
	6	4	2	6	18		−
Berg	119	20	8	78	225	22.4	+
	34	3	2	26	65		−
Föllinge o. Laxsjö	112	28	5	107	252	15.2	+
	23	5	3	14	45		−
Borgvattnet	35	4	1	31	71	14.5	+
	4	1	0	7	12		−
Bräcke	38	14	3	52	107	18.9	+
	11	0	3	11	25		−
Nyhem	38	3	1	31	73	12.0	+
	3	0	3	4	10		−
Sunne	31	9	1	34	75	20.0	+
	7	1	2	5	15		−
Hackås	35	6	4	13	58	20.5	+
	7	1	0	7	15		−
Offerdal	47	14	2	46	109	15.5	+
	9	2	3	6	20		−
Oviken	18	4	1	12	35	18.6	+
	3	0	1	4	8		−
Mattmar	31	8	2	29	70	23.9	+
	11	3	1	7	22		−
Total	1463	364	126	1180	3133	16.7	+
	290	64	39	243	636		−

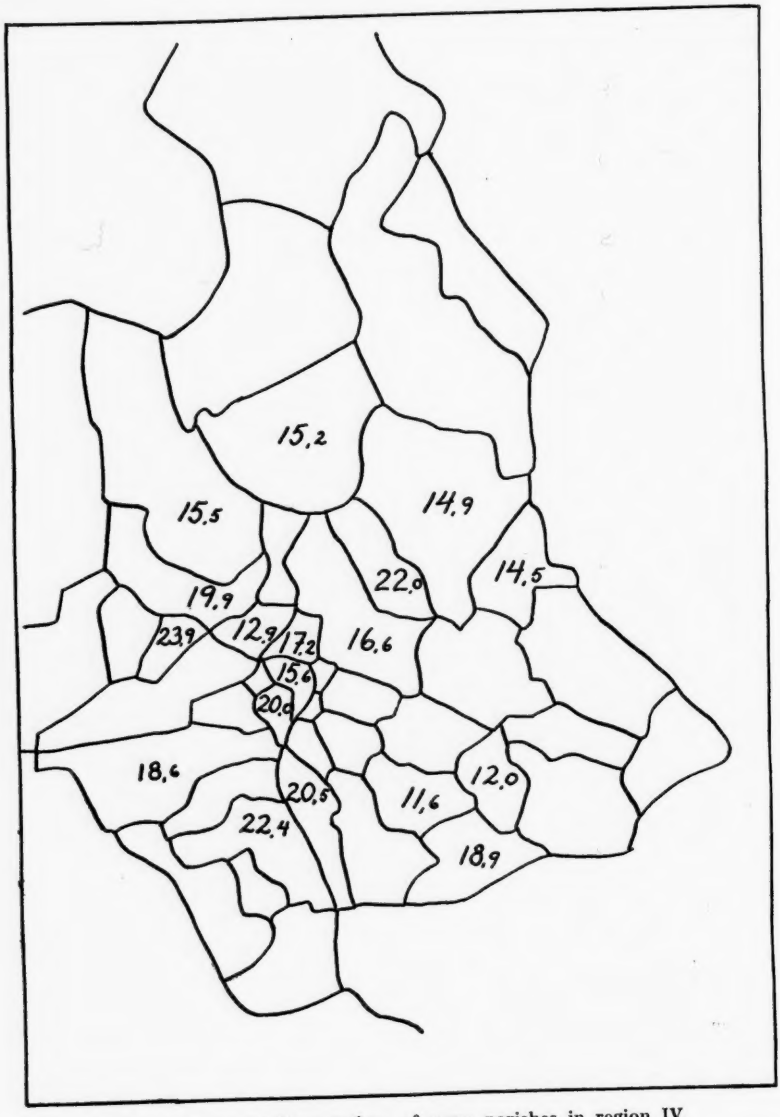

Fig. 5. Per cent Rh-negatives of some parishes in region IV.

degrees of freedom. Thus, there is a significant heterogeneity for the Rh-negatives in the county of Jämtland. The percentages vary between 11.6 and 23.9. The heterogeneity might depend on the mixture of Lappish and Nordic populations since Jämtland is the southernmost county with any considerable amount of Lappish population and, at the same time, has an old stock of Nordic people connected with the Mälar District.

SUMMARY

(1) AB0 blood group data are given for 49.144 conscripts, as well as Rh-data for 3.763 blood donors.

(2) There is a considerable heterogeneity in the geographical distribution of all genes. The A-gene frequencies vary between 19 and 43 per cent, the B-gene frequency between 2 and 15 per cent and the 0-gene frequency between 55 and 75 per cent.

(3) Three main population groups can be distinguished.

a) One group with high 0 and low A and B which probably immigrated from the southwest.

b) One group with high A which probably immigrated from the north.

c) One group with high B which probably immigrated along two main passages from the east.

(4) The distribution of Rh-negatives in the mid-central east region (region IV, see Fig. 5) shows a significant heterogeneity.

(5) No significant correlation between anthropometrical and serological data was found in northern Sweden as a whole.

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STUDIES ON X-RAY INDUCED DETRIMENTALS IN THE SECOND CHROMOSOME OF DROSOPHILA MELANOGASTER

By *GERT BONNIER* and *ULLA B. JONSSON*
INSTITUTE OF GENETICS, UNIVERSITY OF STOCKHOLM, SWEDEN
(Received February 15th, 1957)

THE great majority of experiments with *Drosophila* on radiation induced mutations centers about questions of lethal mutations. Several investigations are, however, also made on mutations with less drastic effects, and it seems as if, during the last decade, the interest has switched over to mutations of this kind. The present investigation deals with different questions concerning x-ray induced subvitals in the second chromosome of D. *melanogaster*. The frequency of such mutations are considered and the comparisons made are in some instances also combined with the effects of different degrees of environmental stresses.

MATERIAL

The wild type flies used in the experiments were extracted from a Canton S stock. The flies were first made co-isogenic by making the crosses outlined in Fig. 1. As may be seen, (matings 1—5) the second chromosome *al*^{2} *Cy lt*^{3} *sp*^{2} (abbreviated *Cy*) was used as marker and crossing over suppressor during the isogenisation. Later it was substituted by the chromosome *al*^{2} *Cy lt*^{3} *L*^{4} *sp*^{2} (abbreviated *Cy L*; Fig. 1, mating 6 and onwards). The matings 1, 2, 3 and 4 were made in several replicates. In one of these the progeny of mating 4 was 26+, 53 *Cy*, 49 *D* 89 *Cy D*, which is in good agreement with the expected proportions 1: 2: 2: 4 (P between 0.7 and 0.8). The parents of mating 5 showed in this replicate good fertility; this replicate was, therefore, used as the foundation stock from which all »wild type» chromosomes of the present study emanate. In a sideline the chromosomes *al*^{2} *Cy lt*^{3} *sp*^{2} and *ru h D*^{2} *Ins CXF ca* (abbreviated *Cy D*) were retained on the male side

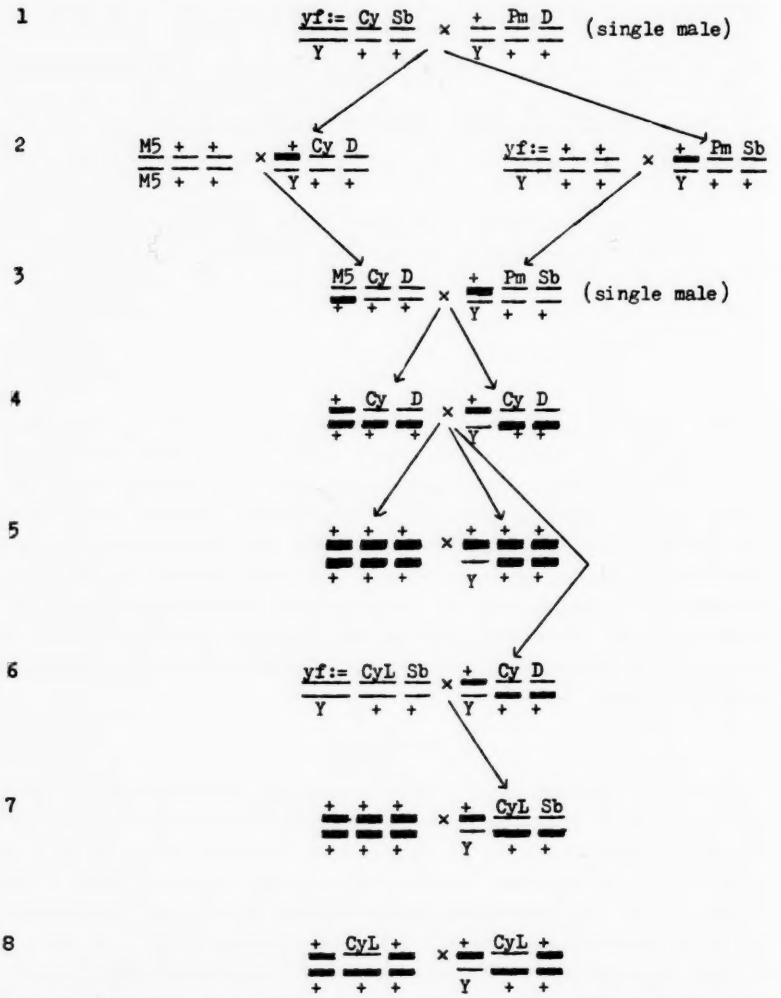

Fig. 1. Isogenisation of the three major chromosomes. Heavy lines indicate chromosomes with regard to which the stocks were made co-isogenic.

(mating 4) for use in connection with the substitution of *Cy L* for *Cy* and for the irradiations.

Two irradiation series were started at two different occasions with a few months inbetween but otherwise in exactly a similar manner (Fig.

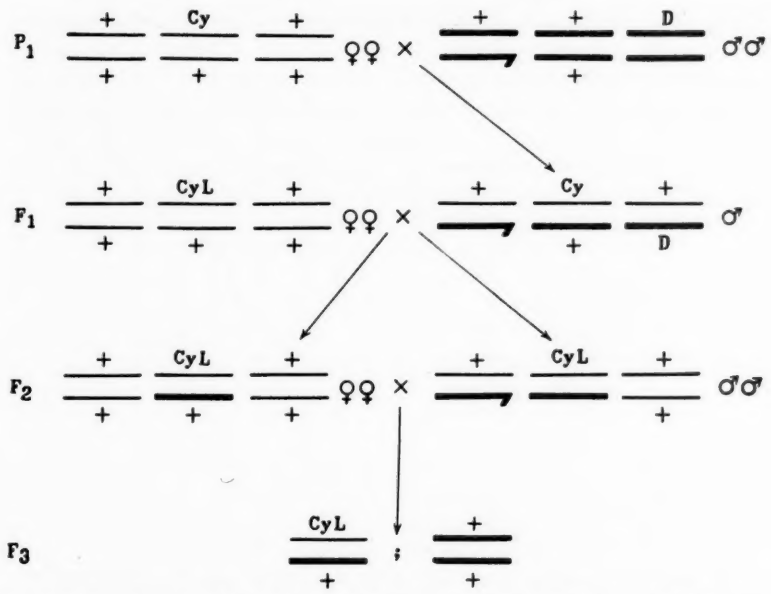

Fig. 2. P 1 males which in their third chromosome were heterozygous for D were given 3000 r from x-rays, and subsequently mated to *Cy* females.
Heavy lines indicate irradiated chromosomes. The diagram shows how the irradiated first and third chromosome were substituted by non irradiated chromosomes. The observed percent of wild type flies in F 3 is in the next named »original survival rate».

2). Thus *D* males of about 24 hours of age were given an x-ray dose of 3000 r. The irradiated males were put into vials, 2 males and a few females per vial. After 2 days these females were killed, the males transferred to new vials with virgin somewhat aged *Cy* females, again 2 males and a few females per vial (Fig. 2, P 1). After 2 more days the males were killed without etherizing the females. *Cy D* sons were then mated singly in vials to virgin *Cy L* females (Fig. 2, F 1). From the progeny within each separate vial virgin *Cy L* females were mated to *Cy L* brothers (F 2). As may be seen these *Cy L* flies had one second chromosome irradiated and the males carried an irradiated *Y*-chromosome. The first and the third chromosomes were, however, unirradiated and no check was kept with regard to chromosome 4. The F 2 matings were also made in vials, usually with 3 females and 2 males per vial and, according to how many virgins there were found, 1 to 3 vials were started. Many of the strains so produced were kept for several generations by

TABLE 1. *Summary of irradiated strains tested by »original» survival rates i. e. by percent wild type flies in F 3. Fig. 2. (For the distinction between semilethals, subvitals and normals, see text and Table 2). Lethals in percent of total; others in percent of total after exclusion of lethals.*

	Irradiation series		Total	Percent
	1	2		
Lethals	13	14	27	15.6
Semilethals	2	1	3	2.1
Subvitals	1		1	0.7
Normals	73	69	142	97.2
Total	89	84	173	

crossing *Cy L*/+ × *Cy L*/+ within the different strains. The percentage of wild type flies among the F 3 progeny will be named »original» survival rates whereas the corresponding percentage from later generations will be named »secondary» survival rates.

In the two irradiation series, taken together, there was produced a total number of 236 *Cy D* males (Fig. 2, F 1). Some of these were sterile, presumably because of breaks in their *Y*-chromosome; others produced no *Cy L* offspring in either sex (2—3 translocation); or *Cy L* only in the female sex (*Y*-3 translocation); or *Cy L* only in the male sex (*Y*-2 translocation). The number of chromosomal strains remaining was 89 in the first and 84 in the second irradiation series (Table 1). Of these 13 and 14 respectively were lethals. As controls there were progenies from 107 matings *Cy L*/+ × *Cy L*/+. Of these 99 were taken from mating 8 Fig. 1 (or from a later mating of a similar kind) and 8 strains which were produced in a similar way as the irradiated ones (Fig. 2), but without any x-ray treatment. From the latter of these controls there are available »original» as well as »secondary» survival rates.

ESTIMATES OF THE FREQUENCY OF SUBVITALS

In any estimate of the frequency of a certain kind of chromosomal strains there must be at hand a standard for comparison. In the many studies made by DOBZHANSKY and WALLACE and their group »random heterozygotes» have been used as such standards. These heterozygotes are produced by crossing *C*/+1 × *C*/+2, where *C* symbolizes a dominant

TABLE 2. *Distribution of percent wild type flies in crosses Cy L/+ × Cy L/+.*

The table includes 99 controls (taken from mating 8 Fig. 1 or from a similar cross in a later generation), and 8 further control strains and 146 irradiated strains. For these two last mentioned groups the figures in the table are original survival rates, i. e. percent wild type flies in F 3 Fig. 2. The grouping unit is $s/2$, where $s=6.06$ percent is the standard deviation of the observed 107 control strains. Central group (with limits 31.2 and 34.2) is placed symmetrically round the average, 32.71, of the controls. »Expected» is the expectation according to the normal distribution which has the average and standard deviation of the controls. Because of later references the 4 irradiated strains with the lowest percent wild type flies are named in the table with their record numbers together with their percent wild type flies.

Percent wild type	7.0	10.0	13.0	16.1	19.1	22.1	25.1	28.2	31.2	34.2	37.3	40.3	43.2	46.4	Total	χ^2	df	P
Controls observed				1	1	2	6	16	13	30	15	10	9	2	2	107			
Controls expected						4.29	7.01	12.95	18.69	21.12	18.69	12.95	7.01	4.29		107	8.3	7	0.3
Irradiated strains observed	1 nr. 3 5.4	1 nr. 119 9.8		1 nr. 68 15.2		1 nr. 58 21.4	3	15	32	48	28	13	3			146			
Irradiated strains expected						5.85	9.56	17.67	25.51	28.82	25.51	17.67	9.56	5.85		146	31.7	6	< 0.0005

marker and crossing over reducer (corresponding to *Cy L* in the present paper) and +1 and +2 are two different wild type chromosomes. The average percentage of wild type flies from a number of such crosses is then by definition taken as the »normal» percentage or »normal» survival rate, which is used as the standard. This kind of comparison has been very successful for the studies of evolutionary questions and of problems of population genetics. As the present paper deals with homozygotes produced, after irradiation, from a common co-isogenic stock of homozygotes, it seems, however, here more appropriate to use this stock, i. e. the controls (Table 2), as standard. The now usual way of classifying viability strains (HADORN 1949) will be adopted here. Strains which produce homozygotes in less than 10 percent of the standard are said to be lethal; if this percentage is between 10 and 50, the strain is said to be semilethal; and when this percentage is above 50 but with »certainty» below 100 the strain is subvital.

Different methods of estimating the frequency of subvitals have been used. In earlier studies DOBZHANSKY et al (DOBZHANSKY, HOLZ and SPASSKY, 1942) constructed the normal distribution with average and variance of the standard. After grouping the observed frequencies of their homozygotes into the classes of this normal distribution they were able to compare these frequencies with the expected ones and used the differences so found as estimates. But by doing so they could not pick out every one of the individual subvital strains but only just to estimate their numbers. Later WALLACE and MADDEN (1953) and DOBZHANSKY and SPASSKY (1953) defined those strains as subvitals in which the survival rate was less than the average of the standard minus 2 times its standard deviation.

KÄFER (1953), who studied x-ray induced viability mutations in the first and the second chromosome in D. *melanogaster*, used in her statistics the so called angular transformation. She defined the subvitals in the common manner but had to recalculate the class limits into angels (see also below, section 5).

The results with regard to the »original» survival rates of the present study are found in Table 2 (in which the two irradiation series are pooled). The text of the table explains the nature of the grouping. It will be seen that there is practically no difference between the averages of the controls and of the irradiated strains. The primary reason for the large value of χ^2 of the irradiated strains is the excess in the central class where 48 strains were observed but only 29 expected. (Also among the control strains there was an excess observed in the central class).

The irradiated strain classified between the limits 19.1 and 22.1 (nr. 58) produced 21.4 percent wild types, thus just on the border line to subvitality. As the females of this strain were sterile it is here classified as subvital. Though there were other strains which were not normal (for instance as being visibles) Table 2 shows that from the point of view of survival rate (percent wild type flies), not more than 4 out of 146 non lethals or 2.8 percent (Table 1) are non normals. It must, however, be remembered that a part of the variation is lost by grouping and it should also be observed that, in computing χ^2 the 2 strains with percents smaller than 19.1 are pooled with the 2 strains belonging to the group with limits 19.1 and 22.1. Concerning the controls they fit to the expected distribution.

PARTITIONING OF THE VARIANCE INTO ITS COMPONENTS

WALLACE and MADDEN (1953) and DOBZHANSKY and SPASSKY (1953) have described methods for partitioning the total observed variance σ^2_o into 3 parts, viz (1) that due to genetical causes σ^2_g (their σ^2_r), (2) that due to environmental causes σ^2_e, and (3) random sampling σ^2_s.

Beginning with the last of these, it is known that, in the case of two alternating characters (as wild type and non-wild type), this variance is pq/n, where p is the proportion of one of the characters, q the proportion of the other, and n the number on which these proportions are based. When all variation is due to random causes p is practically normally distributed if the product of n and p, (p being the smaller of the two proportions) is not too small, say at least equal to 15. Therefore, when determining the proportion of wild type flies from the crosses $Cy\ L/+ \times Cy\ L/+$ where the expected value of p is $^1/_5$, the exclusions of all cultures with n less than 45 or, somewhat better, less than 50 ensures the use of pq/n as the variance of a normal deviate, the deviation being caused by random sampling. In the present study, therefore, all percentages of wild type flies are based on numbers not less than 50 (usually on much larger numbers). When there are several cultures, say h, the best estimate of the sampling variance is the arithmetic mean of the individual variances $S(pq/n)/h$. As WALLACE and MADDEN (1953) point out, the computation of the estimate may, with a very good approximation, be simplified by substituting the averages $\bar{p}$ and $\bar{q}$ for the individual values of p and q. The estimate of the sampling variance to be used is therefore

$$\bar{p}\bar{q}/H$$

TABLE 3. *Analysis of »original» survival rates (Fig. 2) from control and irradiated strains.*

	Number of strains	Total progeny	Wild type progeny	Percent wild type	Average of percents	Sum of squares of percents $S(p-\bar{p})^2$	Sampling variances of percents $\frac{\bar{p}\bar{q}}{H}$	$\chi^2 = \frac{S(p-\bar{p})^2}{\frac{\bar{p}\bar{q}}{H}}$	Degrees of freedom	P
Control	107	10,852	3,563	32.83	32.71	3,875.94	26.26	147.60	106	0.0005
Irradiated strains	146	36,042	11,294	31.34	31.98	3,662.27	12.82	285.67	145	$\ll 0.0005$

where $H=\frac{1}{h}S\left(\frac{1}{n}\right)$ is the harmonic mean of the numbers n. In Table 3 the sampling variances of the 107 control and the 146 irradiated strains are given.

The next step is to estimate σ^2_e, but before discussing this, the following may be said about tests of significance. WALLACE and MADDEN (1953) determine the significances of σ^2_e by making a χ^2 test of heterogeneity among replicated cultures. The most straightforward way seems, however, to be to divide the estimate of σ^2_e by the estimate $\bar{p}\bar{q}/H$ of the sampling variance. This is distributed as χ^2/degrees of freedom. Therefore one may directly take the sum of squares corresponding to the estimate of σ^2_e and divide it by $\bar{p}\bar{q}/H$ which is a χ^2 with the number of degrees of freedom of the estimate of σ^2_e.

σ^2_e is estimated from replicated cultures between which there is supposed to be no genetical differences. But replicates may, in the kind of experiments dealt with here, be made in two different ways. Parents of cultures may be transferred one or more times to new bottles or vials. The variation from the counts of the different bottles corresponding to the same group of parents is wholly due to environmental causes, at least when there is only one pair of parents. Replicates may also be made by using subcultures, i. e. with new parents which belong to the same strain and, therefore, are looked upon as genetically identical. As usually not all chromosomes are checked, the subculture method may include some residue of genetic origin; σ^2_e may thus be divided into two compounds viz. σ^2_{ee}, which is wholly due to environmental (»experimental») causes, and σ^2_{eg} which may in part be due to environmental and in part to genetical causes. The true genetical part of the variance is finally found by subtracting the sum $\sigma^2_{ee}+\sigma^2_{eg}+\sigma^2_s$ from the observed total variance σ^2_o.

To estimate separately the two parts σ^2_{ee} and $^2\sigma_{eg}$ of σ^2_e the following experiments were made. From within each strain included in the experiment a number of pair matings were made by crossing a *Cy L*/+ female to a brother of the same kind. The pairs were mated in vials in which the females were allowed to oviposit for 5 days. On the 5th day the pairs were transferred to fresh vials, where they remined for 4 days. This experiment was started after the »original» survival rates of the 2nd irradiation series were at hand. At that time most of the strains of the 1st irradiation series were already discarded, only 10 of them were retained (and among these the subvital strain nr 58, see Table 1) for special purposes. Likewise the 8 control strains produced

in the same manner as the irradiated ones were retained so that the experiment did finally include 8 control strains, 10 selected strains of the first irradiation series and all the 70 non lethal strains (including the subvital strain 119, see Table 2) of the second irradiation series. As the averages of the controls and the irradiated strains are practically identical (Table 4), the 88 strains will, in the analysis, be taken together.

TABLE 4. *Progeny from pair matings. The table includes the total numbers, regardless of how many offspring the individual pairs have produced.*

	Number of strains	Total offspring	Wild type offspring	Percent wild type
Irradiated strains	80	108,622	34,746	31.99
Controls	8	10,553	3,380	32.03
Total	88	119,175	38,126	

From each of the 88 strains 14 pairs were usually started, and given record numbers of their own. Of these the 10 first numbers which gave progeny in both of the two replicates (or less if there were not as many as 10 of this kind) were counted, while the rest was discarded. However, in the analysis, only such pairs are included which produced at least 50 offspring in both of the two replicates. Table 5 gives the number of pair matings which fulfilled this requirement and in Table 6 the analysis of variance is shown.

As the mean squares »within strains between pairs» and »within strains within pairs» are of the same order of magnitude as the sampling variance, we may conclude that *in this material* both σ^2_{eg} and σ^2_{ee} are equal to zero. The differences between strains are highly significant, but, as the table shows, this is wholly, or to a very great extent, due to the two subvital strains included in the analysis. After removal of these two strains, also σ^2_g will be not far from zero and most of the remaining variation is wholly due to sampling.

There is another point which may be of some interest. It is well known that changes in environment may change the viability (see for instance TIMOFÉEFF-RESSOVSKY 1935, KERKIS 1938, KÄFER 1953). Our original survival rates were based on tests in vials with usually 3 females per vial, whereas there of course was only one female per vial in the pair culture tests. In many strains the effect of this difference

TABLE 5. *Pair matings which produced at least 50 offspring in both of the replicates.*

Number of pair matings	1	2	3	4	5	6	7	8	9	10	Total strains	Total pairs
Number of strains	1	3	7	11	8	4	11	21	14	8	88	587

TABLE 6. *Analysis of variance of the pair matings of Table 5. Figures in parenthesis refer to numbers after exclusion of the two subvital strains (see Table 2 and text).*

	Degrees of freedom	Mean squares
Between strains	87 (85)	68.23 (33.75)
Within strains, between pairs	499 (489)	27.98 (28.17)
Within pairs, between replicates	587 (575)	29.65 (29.26)
Sampling variance		28.91

Ratios $\frac{68.23}{27.98}=2.4$ $P<0.0005$ $\frac{33.75}{28.17}=1.2$ $P>0.1$

$$\chi^2=\frac{587\times 29.65}{28.91} \quad P>0.3$$

was that the percent of wild type flies were higher in the pair cultures than in the cultures with 3 females per vial. It may, however, be asked how closely correlated these two pairs of percents are.

If we define »secondary» survival rate as the percent of wild type flies found from the total pair mating progeny of the different strains (corresponding to Table 4) then we have 88 pairs of original (p_1) and secondary (p_2) survival rates. The correlation between them is 0.35 which is highly significant ($P<0.001$). But already after exclusion of only that subvital which has the lowest percent wild type (strain 119, $p_1=9.8$, $p_2=19.2$), the remaining 87 strains have the very reduced correlation 0.085 which lacks significance ($P>0.3$).

DISCUSSION ON SURVIVAL RATES

The »original» survival rate is in the present study defined as the percent of wild type flies in F 3, Fig. 2; and the »secondary» survival rate as the percent of wild type flies from a similar cross, though from a later generation. Now, the results described in this paper do not tally well with those of most other investigators. We may for instance

compare the findings of this paper with those of KÄFER (1953). She produced a large material in which she studied viability mutations simultaneously in the first and in the second chromosome. She used three different x-ray doses, of which the lowest, named T1, was »in der Grössenordnung von 2500 r». The two other doses were much higher, and, as we used 3000 r, the comparison will be confined to KÄFER's results with the dose T1. With regard to the second chromosome she grouped the F 3 progeny according to the number of *Cy* flies in the F 3. A corresponding grouping was not made in the present study. As is seen from Table 7, the frequencies of lethals are concordant in the two studies, and the frequencies of semilethals not too discrepant. The subvitals, however, have a very much lower frequency in the present study than in that of KÄFER. It may also safely be said that our frequency of subvitals is unusually low in comparison with the results of most other studies.

TABLE 7. *Comparison between present study and* KÄFER (*1953*) *of percent of different types of viability mutations in the second chromosome. Lethals in percent of total. Others in percent of total after exclusion of lethals.*

	Present study dose 3000 r	From KÄFER dose about 2500 r Number of Cy flies in F 3		
		20—100	101—200	>200
Lethals	15.6	15.8	20.4	14.7
Semilethals	2.1	8.9	2.8	3.8
Subvitals	0.7	15.8	13.9	8.2
Normals (including supervitals)	97.2	75.2	83.3	86.6

When trying to find the explanation of the discrepancy there are two points which should be kept in mind.

(1) If one makes the irradiation as shown in Fig. 2 all major translocations are eliminated. But if one follows the more usual way and irradiates wild type males, such translocations will not be eliminated nor will they be observed. Hence, if there is an influence on the viability from the translocations *per se*, or if there is an interaction between viability mutations in the chromosome studied and in the translocated chromosome, the method of irradiating wild type males could give a higher proportion of subvitals than what we have found. Moreover,

when making crosses in order to produce new generations, *i. e.* to go from »original» to »secondary» survival rates, these procedures will not expel the translocations.

(2) Even without translocations there may, by interactions, be an enhanced frequency of subvitals in F 3. By irradiating wild type males there will be produced F 2 flies having irradiated 3rd chromosomes besides their irradiated 2nd chromosomes, but this will not be the case when following the method shown in Fig. 2. Denote by *AA* flies which have a pair of non-irradiated 3rd chromosomes, and by *Aa* flies with one non-irradiated and one irradiated 3rd chromosome. By irradiating wild type males there will in F 2 be equal numbers of *AA* and *Aa* flies. With only a very limited number of F 2 parents of the different F 3 progenies these parents may range from all being *AA* to all being *Aa*. Prosecuting, after F 3, the strains with a limited number of parents per generation, some of the strains may by drift finally be *AA* and others *aa*. If the strains are prosecuted in large populations they will stabilize — or more correctly — individual loci of the 3rd chromosome will stabilize on 9 *AA*: 6 *Aa*: 1 *aa*. Thus if there are interactions between irradiated 3rd chromosomes and the irradiated 2nd chromosome which is under study, there would probably be observed a higher frequency of subvitals when irradiating wild type males than when following the scheme of Fig. 2.

FALK (1955) studied the effects of irradiating the third chromosome with 2000 r. The method of the present study (Fig. 2) is in principle the same as that used by FALK. But according to his estimates about 75 percent of the strains were affected. Now, the character which was the subject of his study was the hatchability of the eggs; our experiments and his are, hence, not quite comparable. And, as a matter of fact, several of our strains — though showing in F 3 a percent of wild type flies larger than subvitals should do according to the definition of subvitality — were non-normal in other respects. Among other things their rate of development had a larger variance than the »true» normals (BONNIER and JONSSON 1957).

With no other evidences at hand we assume, though quite tentatively, that the reason for the low rate of subvitals observed in the present experiment is that the strains are, so to say, purified from interactions due to irradiation of the residual chromosome set.

LARVAL COMPETITION TESTS

Investigations on larval competition in *Drosophila* have been made in the study of relative viabilities. WALLACE (1952), compared a number of different methods for estimating the adaptive values of his irradiated populations and among these methods he also used results from larval competition tests. VETUKHIV (1954), who studied the importance of heterozygosity in geographical wild populations of D. *willistoni, pseudoobscura* and *paulistorum* likewise based his studies on larval competition tests. Still more recently LEWONTIN (1955) made, by larval tests, an interesting inquiry of differences in viability between a number of homozygotes and heterozygotes from Dr. Wallace's irradiated populations. In populations experiments now under way (see section 7 of the present study), one of the genetic tests is based on larval competition. The experiments to be described here were made as preliminaries to these population experiments, hence, not only for the analysis *per se* of the strains in question, but also as studies in methodology. After a number of different trials we are now using the following technique.

The flies which are used as parents are kept in the incubator room at 25° C. Egglaying on food blackened by charcoal is usually confined to the time from 5 p. m. to 9 a. m. so that the females are allowed to oviposit during 16 hours. After 24—28 hours just hatched larvae are collected (at room temperature) with the aid of a small brush, and transferred to vials 28×90 mm with ordinary corn meal agar food. The amount of food in the different vials is not measured as this would take too much time and as the small variations certainly are without greater importance. The amount of food is thus looked upon as being identical in all vials. In the routine work 800 larvae are collected. Of these 400 are transferred to 16 vials with 25 larvae in each and the further 400 larvae are transferred to 2 vials with 200 larvae in each. Immediately after this transfer the vials are brought to the incubator room. In the tests to be described 28 selected strains were included of which 2 belong to the first, and 16 to the second irradiation series (Table 1). In these cases the larvae were produced in the following way. Virgin *Cy L/Pm* females were crossed to *Cy L/+* males of the different strains (which as already mentioned were kept in stock by mating *Cy L/+* × *Cy L/+*); or, vice versa, virgin *Cy L/+* females of the strains were crossed to *Cy L/Pm* males. From these crosses virgin *Cy L/+* daughters were mated to *Pm/+* brothers, and their larval offspring collected.

It was planned to combine these studies with checks on rate of development. But with egglaying confined to 16 hours, counts of emerged adults should probably be made with rather small time intervals in order to be reliable, and this could not be done. It may, however, be stated that the time from oviposition to eclosion in the 200 larvae vials was round 24 hours longer than in the 25 larvae vials.

The hatching flies were classified both with regard to sex and to the 4 phenotypic classes *Cy L Pm*, *Cy L*, *Pm*, +. In certain strains there were some difficulties to distinguish *Pm* from wild type (this seems to happen, when, as in the present case, the original stock is derived from Canton S). The errors from this source are, however, certainly small.

For 2 of the strains a more detailed study was made (BONNIER, 1957). These strains were nr. 23, with »original» survival rate=26.4; and nr. 58, with »original» survival rate=21.4. (This strains is in Table 2 classified as subvital). With regard to both of these strains it was known

TABLE 8. *Larval competition tests of 2 strains under different selection pressures. (The collecting of the emerged flies were not made at exactly the same hours of the day; the time from egglaying to eclosion can, therefore, not be compared in detail).*

Strain	Number of larvae per vial	Eclosion, days from egglaying	Cy L Pm	Cy L	Pm	+
58 original, survival rate: 21.4 %	25	10 or less	100	91	89	43
		11—12	4	9	1	20
		13 or more	—	1	—	—
		Total	104	101	90	63
	200	10 or less	77	94	90	—
		11—12	14	6	7	35
		13 or more	—	—	2	12
		Total	91	100	99	47
23 original survival, rate: 26.4 %	25	10 or less	12	29	27	6
		11—12	91	57	70	51
		13 or more	8	1	—	14
		Total	111	87	97	71
	200	10 or less	—	4	2	—
		11—12	59	64	80	43
		13 or more	39	21	10	11
		Total	98	89	92	54

TABLE 9. *Analysis of heterogeneity of adults in larval tests.*

Number of larvae per vial		Females				Males				Dead	Total	χ^2	df	P
		Cy L Pm	Cy L	Pm	+	Cy L Pm	Cy L	Pm	+					
25	Total observed	1,435	1,462	1,359	1,236	1,342	1,333	1,237	1,188	608	11,200	529	216	$\ll 0.001$
	Average of 28 strains	51.3	52.2	48.5	44.1	47.9	47.6	44.2	42.4	21.7	400			
200	Total observed	1,292	1,314	1,310	1,088	1.335	1,385	1.187	1,140	1,149	11,200	845	216	$\ll 0.001$
	Average of 28 strains	46.1	46.9	46.8	38.9	47.7	49.5	42.4	40.7	41.0	400			

that the wild type flies showed a retarded development (BONNIER and JONSSON 1957). Table 8 shows the result. The wild type flies of strain 23, which had a somewhat higher survival rate than strain 58, developed fairly well under low selection pressure (25 larvae per vial) but were more retarded under high selection pressure (25 larvae per vial). In the case of strain 58 the difference in rate of development and survival rate was still more pronounced.

The results with regard to all the 28 strains which were used in these tests are very different for the different strains. Table 9 shows in abbreviation a χ^2 test of heterogeneity. As the numbers of dead are *observed* frequencies they must be included in the analysis, but as they are found as the difference between the fixed total 400 and the observed total, they cannot be specified as to sex. The analysis is hence based on a table with 9 entries for each of 28 rows, why the degrees of freedom are $8 \times 27 = 216$. As the table shows there is a very pronounced heterogeneity. This heterogeneity is the statistical manifestation of the different ways in which the wild type flies of different strains react to changes in environmental stresses.

CONSIDERATIONS CONCERNING THE STUDY AND THE STRUCTURE OF IRRADIATED POPULATIONS

During the last decade our outlook on the structure of populations has undergone far reaching changes. This is, as is well known, primarily due to the pioneer work by TH. DOBZHANSKY and his co-workers on natural populations. And from the very important studies by WALLACE et al on experimental populations under irradiation, it is known that these populations adjust themselves to a state very similar to that occurring in natural populations. The opinions of different scientists seem, however, not to be unanimous when questions are discussed concerning how much of these results from *Drosophila* investigations may be extrapolated to the human field. The controversial point is about the strength of the selection pressure. In the irradiated populations of Dr. B. WALLACE there must be a very strong competition between the growing larvae, leaving only the most viable ones to reach adult age. And such a competition, it must be supposed, does not occur in human populations, at least not in the western so-called welfare communities.

With such considerations in mind combined with the experience that we have gained on larval competition tests we have started experi-

ments on irradiated populations. In these the populations are, so to say, renewed for each generation. Larvae, just hatched from eggs laid by flies belonging to a certain generation, are collected and transferred to vials: in one population each vial contains 25 larvae; and in a second populations each vial contains 200 larvae. 4000—5000 larvae collected in this way constitute the succeeding generation. So far, only a few generations are produced, and detailed descriptions must await results from some more generations. In a personal discussion between the senior author and Dr H. MULLER in August 1956, Dr. MULLER stressed that, in order to make the population experiments more adequate for extrapolations to the human field, one must also have a guarantee that each separate female contributes only a progeny of a very limited size of the next generation. We are, in our experiments, trying to pay regard also to this point of view.

One of the most important findings in the work of the DOBZHANSKY—WALLACE school is that a large majority of population chromosomes are lethal or subvital in homozygous condition; and this is true in spite of the fact that the populations, as such, may show a very good viability. To explain this, it has been supposed that all chromosomes have a large number of loci with viability genes and that for each locus there is a large series of multiple alleles. If in such a population the frequency of homozygous zygotes is low, the individual flies would be heterozygous for most of the viability loci. It is easy to construct a corresponding mathematical model. Suppose that, in a certain locus, there are n alleles a_1, a_2, ... a_n, with the frequencies p_1, p_2, ... p_n. The distribution of homo- and heterozygotes would then follow the generalized HARDY-WEINBERG formula $(p_1+p_2+\ldots+p_n)^2$. If there is one »type» gene, say a_1, then one would have to suppose that p_1, is larger than, or at least, not much smaller than $^1/_2$. But suppose that all alleles a_1, a_2, ... a_n have low frequencies. For simplicity we may suppose that $p_1=p_2=\ldots=p_n=1/n$. The frequency of homozygotes with regard to a single of the alleles is $1/n^2$. As there are n alleles the total frequency of homozygotes will consequently be $1/n$. This means that, if n is large, the incidence of homozygotes will be small. In our population experiments we are planning to study this question as well.

Acknowledgement. — This work was supported by a grant from the Swedish Research Council of Natural Science.

SUMMARY

(1) A study is made of x-ray induced viability mutations in the second chromosome of *Drosophila melanogaster*. Males of a co-isogenic stock were given 3000 r; In the subsequent crosses non-irradiated first and third chromosomes were substituted for the irradiated ones. 173 irradiated strains were produced in this way and for comparison 107 control strains were used.

(2) The viability of the different strains was measured by the survival rate, i. e. the percent wild type flies produced from the cross *Cy L*/+ × × *Cy L*/+. When the counts were made in the F 3 progeny (Fig. 2) the survival rate was named »original»; when the counts were made in a later generation the survival rate was named »secondary».

(3) 27 (15.6 %) of the 173 irradiated strains were lethal. From a study of the distribution of the original survival rates of the remaining 146 strains it was found that 3 (2.1 %) were semilethals and only 1 (0.7 %) subvital. With regard to the frequency of lethals these findings are in good agreement with results of other investigators. But the results are very discordant with regard to the frequency of subvitals. As the only difference in methods used, is the substitution, in the present study, of non-irradiated first and third chromosomes for the irradiated ones, it is tentatively supposed that it is the lack of interactions from these chromosomes which is the reason for the low frequency of subvitals.

(4) The partitioning of the variance into its components is discussed. Using several of the strains, a number of pair matings were made within each of the strains, and each pair mating was replicated. In this way the observed variance could be partitioned in four parts: true genetical, environmental with a genetical residue, pure environmental, random sampling. A direct method was used for testing the significance of the environmental part of the variance.

(5) The secondary survival rate found from the pair cultures are compared with the original survival rates. A significant correlation is found, but after exclusion of the strain with the lowest survival rate no correlation is left.

(6) Larval competition tests were made for a number of the strains. In these tests two degrees of selection pressures were used; fresh larvae were transferred to vials, in one set there were 25 larvae per vial and in another set 200 larvae per vial. The results were very heterogenous for the different strains. The wild type flies in two strains which had a

low survival rate, emerged in very different proportions under these two degrees of selection pressures.

(7) With regard to the usefulness of such graded selection pressures experiments on irradiated populations are started. New generations are in these produced by larvae which in one series grow in vials with low pressure and in another series in vials with high pressure. Problems and experiments in connection with such studies are briefly discussed.

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BRIEF REPORTS

A. LIMA-DE-FARIA: *Goldschmidt's* interpretation of the gene concept and the problem of chromosome organization.

(Received March 1st, 1957)

At the turn of the century, physics had reached an enormous development comparative to the other neighbouring sciences which resulted in a strong impact of physics on biology. Most phenomena then seemed to be easily explained in terms of the Newtonian concepts of matter, gravitation, space and time. Both a mechanical and a mechanistic interpretation of natural phenomena dominated physics. The impact on biology which was then searching for an experimental basis for its further development, was very large and fruitful. Later on, physics kept the mechanistic interpretation but exchanged the strictly mechanical approach for a conception based mainly on structural organization and on interrelations among the different components of a system. But genetics which was born at the dawn of the century assimilated the ideas flourishing at the time and embarked with them in its study of inheritance. Since this approach which culminated in the work of the MORGAN school and in the neo-Darwinistic evolution theory was most fruitful, it has established itself so deeply in the minds of most geneticists that, without being aware, they are today still employing concepts based on the philosophical outlook of XIX century physics.

It is frequent to hear among qualified geneticists that chromosome movements cannot be explained except in terms of attraction or repulsion »forces» (by which they mean the concept of a NEWTON vector) or to interpret most variations of the properties of chromosomes by a simple mechanical deformation of their components. A typical example of the same Newtonian mechanical way of approaching genetic phenomena within the gene theory is, for instance, the Treffer theory. Genetics and cytology are still so pervaded by this interpretation that when geneticists find opposition to this way of thinking they believe that the only alternative to a mechanical approach is vitalism or some kind of idealism.

The picture is, however, a different one. In the last 50 years, biochemistry has developed as quickly as genetics. The findings obtained in this science together with the new developments in physics furnish a new approach to genetical and cytological problems. This conception lays its greatest emphasis on spatial organization and chemical interactions, that is, on pattern and function.

RICHARD GOLDSCHMIDT in his new book »Theoretical genetics» shows that he is among the few that has kept pace with the developments of his own science and is trying to take the best advantage of today's tools. To see this attitude coming from Professor GOLDSCHMIDT is most stimulating and in a way surprising since he is among the oldest of the living geneticists and one of the few alive who has seen genetics being born (in 1904 he had already studied the function of chromatin).

The two concepts of spatial arrangement and functional interdependence are the central theme of GOLDSCHMIDT's interpretation of genetic and cytological phenomena. The development of these ideas results in a very critical treat-

ment of many problems within cytology and genetics such as chromosome structure and organization, chromosome chemistry, the gene concept, cytoplasmic inheritance, genic action, sex determination, and evolution of the genic material. The central question which GOLDSCHMIDT has emphasized for several years is again repeated here in various forms, such as: »In my view of the genic material, there is no corpuscular gene but only a definite pattern in the chromonema the changes of which are the mutations.»

It is true as GOLDSCHMIDT points out, and he makes of the following two statements his most important arguments, that (1) the gene is only known through mutation »the mutation creates the gene», and (2) all mutations would probably turn out to be position effects if the structural analysis of the chromosomes could be carried out below the microscopic level. Based on these two arguments, he concludes that there is no evidence for the existence of the classical gene. But these arguments — and here we arrive at what I think is one of the weakest points in GOLDSCHMIDT's way of reasoning — do not contradict the existence of the gene, they only expose the limitations of the methods so far available for its study.

Two other no less important findings can be opposed — which GOLDSCHMIDT does not consider — and they argue equally for the existence of the gene. First in importance are the fundamental findings of the MORGAN school, (1) that not all regions of the chromosome were found responsible for the formation of the same kind of characters and (2) that these regions behaved to a large extent independently of each other. This differential behaviour may depend on a reorganization of the elements at the molecular level, but the different segments do not reorganize in the same way since a certain segment may have, for instance, its predominant effect on eye colour and another on wing venation. The last fifty years' work, using the crossing method, shows irrefutably that the chromosome is differentiated into segments which are not all responsible for the same type of functions and that these functions are usually maintained when the segments change position within the chromosome complement. It is true that the same gene affects different characters and that the same character is affected by several genes, but this does not conflict with the degree of specialization found for most genes.

The second argument is cytological. The chromosome is known, not to be an undifferentiated continuum, but a body divisible into regions with well defined functions: kinetochore, telomeres, nucleolar organizing region, chromomeres, etc. All these regions have specific properties and functions and a definite structural pattern. And, like the genes, these segments keep their essential properties when moved to new regions within the chromosome complement.

Thus, a fundamental acquisition of genetics is both the genetical and cytological evidence of a specialization in function within the chromosome body confined to definite segments. These specialized definite segments are what JOHANNSEN called genes for the sake of simplicity, and what we still call genes for the same reason. Whether they will be as long as a crossing over unit as thought in MORGAN's time or whether their barriers will not be easy to define as pseudoallelism suggests, does not impair the validity of the essential part of the concept.

The question may now be asked, whether the existence of the gene is in-

compatible with the concept of the chromosome as a functional unit, or with the recent evidence obtained from cytology of the existence of a definite pattern in the arrangement of the chromomeres at pachytene? Here, I think, we come to the central contradiction in GOLDSCHMIDT's way of reasoning. He simply equates the concept of the chromosome as a functional unit with that of a genetic unit. This is done throughout the book. By denying the gene concept, he considers the chromosome to be the genetic unit, but most of the time he refers to the chromosome as a functional unit. These two ideas are fundamentally different since the first assumption is incompatible with the gene concept while the second is not. The concept of the chromosome functioning as a whole with its regions interacting in an hierarchic way — a thought that Professor GOLDSCHMIDT also defends — demands the existence of distinct specialized regions which are genes or aggregates of genes. Without such differentiation, a balanced system can hardly be conceived. The cytological evidence available at present clearly points to the predominant role of the kinetochore in chromosome organization interacting with other specialized regions of the chromosome, such as telomeres and nucleolar organizing regions, which would have minor hierarchic roles in the maintenance of these interactions. Such evidence does not, however, conflict with the gene theory; on the contrary, as I have pointed out recently, it demands it.

Although it may seem surprising, GOLDSCHMIDT draws very few examples from the genetic work as a basis for his arguments on chromosome organization. He, instead selects them from cytology, emphasizing the significance of the chromomere size gradients which I described in rye, *Agapanthus*, and other species; and the constancy of the karyotype in certain species groups. The reason for this may be found in the fact that he has not realized, as many others, that most known genes were studied by their effect on the organism phenotype and not on the chromosome phenotype. All the position effects, referred to in his previous reviews, which were written to show that the gene concept should be discarded, could not led him to a better understanding of the chromosome organization since these position effects were known by the expression of their properties at the organism and not at the chromosome level. When analysing MCCLINTOCK's and RHOADES' recent work in maize which furnishes the best genetic evidence for the understanding of the organization of the chromosome, he still has not been able to realize the distinction between the two levels of gene expression with the result that he could not reach a coherent picture of the organization of the chromosome.

It is, thus, not without fundamental reason that GOLDSCHMIDT's point of view has received such strong opposition from most geneticists. He tried to destroy a concept and instead offered a new one for which he lacked a sound basis. However, by introducing the modern way of thinking, he has done a large service. It would be very harmful for the future development of genetics if these two points of view would become associated in the mind of most geneticists. At present, the evidence is growing rapidly that the spatial organization of the different elements within the chromosome and the interactions between them are leading us to a new concept of the chromosome. This approach will not reject the gene theory but will lead it into new pathways.

This criticism should not divert the student of genetics from carefully

reading this book. It contains a very brilliant and stimulating analysis of the modern findings within cytology and genetics which can hardly be found in any other recently published work.

Institute of Genetics, University of Lund, Sweden.

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OLLI HALKKA: A note on the chromosome numbers in the *Homoptera Auchenorrhyncha*. (Received November 2nd. 1956)

The following chromosome counts have been made on leafhopper material collected in the years 1949—1951 and 1953—1956 in various localities in South-western Finland. With two exceptions, formed by *Aphrophora forneri* and *Empoasca apicalis,* the nomenclature and the sequence of families are those used by OSSIANNILSSON (1946—1947). The counts have been made almost exclusively from sectioned material, fixed in BOUIN-ALLEN-BAUER, BENDA or CARNOY, and stained in FEULGEN, and in some cases also in HEIDENHAIN's haematoxylin. In addition, acetic alcohol FEULGEN squashes have been made. For every species, the number of chromosomes (bivalents and sex chromosome univalents when present) observable in the first spermatocyte division has been given. A note on the sex chromosome system has been enclosed in brackets. If any uncertainty exists as regards the sex chromosome system, a question-mark (?) follows the note. There are 90 species included in the list. Certain of the species (at least *Philaenus spumarius, Neophilaenus lineatus* and *Tettigella viridis*) have been studied by earlier authors.

ARAEOPIDAE: *Kelisia ribauti* W. WAGN. 15 (XO?), *Megamelus notula* GERM. 14 (XO), *Araeopus crassicornis* PANZ. 15 (XO), *A. pulchellus* CURT. 15 (XO), *Chloriona smaragdula* STÅL 17 (XO?), *C. glaucescens* FIEB. 16 (XO?), *Criomorphus affinis* FIEB. 15 (XO?), *Dicranotropis hamata* BOH. 15 (XO?), *Calligypona pellucida* F. 15 (XO), *C. forcipata* BOH. 15 (XO), *C. elegantula* BOH. 15 (XO), *C. pallens* STÅL 15 (XO), *C. sordidula* STÅL 15 (XO), *C. lugubrina* BOH. 13 (XY?), *Conomelus limbatus* F. 12 (XO), *Euconomelus lepidus* BOH. 15 (XO). — ISSIDAE: *Ommatidiotus dissimilis* FALL. 13 (XO). — CERCOPIDAE: *Lepyronia coleoptrata* L. 11 (XO), *Aphrophora alni* FALL. 15 (XO), *A. forneri* HPT. 15 (XY), *Philaenus spumarius* L. 12 (XO), *Neophilaenus exclamationis* THUNB. 10 (XY), *N. lineatus* L. 15 (XO). — EUPELICIDAE: *Eupelix depressa* F. 9 (XO). — EUACANTHIDAE: *Euacanthus interruptus* L. 13 (XO). — PROCONIIDAE: *Tettigella viridis* L. 10 (XO). — MACROPSIDAE: *Macropsis fuscula* ZETT. 11 (XO), *M. impura* BOH. 11 (XO), *Oncopsis flavicollis* L. 10 (XY), *O. tristis* ZETT. 11 (XO), *O. alni* SCHRNK. 10 (XY?). — IDIOCERIDAE: *Idiocerus lituratus* FALL. 12 (XO), *I. elegans* FL. 12 (XO?), *I. populi* L. 12 (XO), *I. confusus* FL. 12 (XO). — AGALLIIDAE: *Agallia venosa*

FALL. 11 (XO). — TYPHLOCYBIDAE: *Dikraneura aureola* FALL. 11 (XO), *Notus flavipennis* ZETT. 9 (XO), *Empoasca smaragdula* FALL. 10 (XO), *E. apicalis* FL. *sensu* NAST 9 (XO), *Cicadella pictilis* STÅL 8 (XO), *C. pulchella* FALL. 6 (XO), *C. concinna* GERM. 10 (XO), *C. cyclops* MATS. 9 (XO), *Typhlocyba douglasi* EDW. 10 (XO), *T. ulmi* L. 9 (XO), *Erythroneura rubrovittata* LETH. 11 (XO), *E. alneti* DAHLB. 12 (XO). — EUSCELIDAE: *Aphrodes bifasciatus* L. 12 (XO), *A. bicinctus* SCHRNK. 11 (XY?), *Strongylocephalus agrestis* FALL. 10 (XY), *Macrosteles sexnotatus* FALL. 9 (XO), *M. viridigriseus* EDW. 9 (XO), *M. fieberi* EDW. 9 (XO), *M. horvathi* W. WAGN. 9 (XO), *Balclutha punctata* THUNB. 9 (XO), *Graphocraerus ventralis* FALL. 9 (XO), *Doratura stylata* BOH. 10 (XO), *Paramesus nervosus* FALL. 9 (XO), *Paralimnus phragmitis* BOH. 10 (XO), *Scaphoideus marmoratus* FL. 9 (XO?), *Psammotettix confinis* DAHLB 9 (XO), *P. poecilus* FL. 9 (XO?), *Deltocephalus pulicaris* FALL. 10 (XO), *Sorhoanus assimilis* FALL. 9 (XO), *Arthaldeus pascuellus* FALL. 9 (XO), *Diplocolenus abdominalis* F. 9 (XO), *Lausulus flori* FIEB. 9 (XO), *L. allobrogicus* RIB. 9 (XO?), *Cosmotettix edwardsi* LINDB. 8 (XO?), *C. panzeri* FL. 8 (XO), *C. costalis* FALL. 9 (XO), *Limotettix striola* FALL. 6 (XO), *L. atricapilla* BOH. 6 (XO), *L. striatulus* FALL. 6 (XO), *Streptanus aemulans* KBM. 9 (XO?), *S. marginatus* KBM. 10 (XO), *Athysanus argentatus* F. 9 (XO), *Allygus mixtus* F. 7 (XO), *A. commutatus* SCOTT 10 (XO), *Macustus grisescens* ZETT. 8 (XY), *Thamnotettix confinis* ZETT. 9 (XO), *Solenopyx sulphurellus* ZETT. 8 (XO), *Cicadula intermedia* BOH. 8 (XO), *C. quadrinotata* F. 8 (XO), *C. saturata* EDW. 8 (XO), *Rhopalopyx preyssleri* H.-S. 8 (XO), *R. flaveolus* BOH. 8 (XO), *Speudotettix subfusculus* FALL. 10 (XO), *Coryphaelus gyllenhali* FALL. 9 (XO).

The list includes two cases in which two species belonging to the same genus have widely different chromosome complexes. In the Cercopid genus *Neophilaenus, N. lineatus* has 14 bivalents and a univalent X-chromosome, while *N. exclamationis* has 9 autosome bivalents and a sex chromosome bivalent. Again, in the family Typhlocybidae, *Cicadella concinna* has the haploid chromosome number 10, while *C. pulchella,* which is very like it both morphologically and as regards its habit of life, has only 6 chromosomes in the haploid set. It is a general rule that the larger the number of chromosomes, the smaller is their size. The smallest chromosomes have been found in the family Araeopidae, the largest among the Euscelidae in the genus *Limotettix.*

At least in one case a »fusion» between the X-chromosome and an autosome bivalent seems possible. In *Athysanus argentatus* there are 8 bivalents and an X-chromosome univalent in the first spermatocyte plate. In *Macustus grisescens,* a closely related species, there is a large XY-pair among the bivalents at this stage, the total number of bivalents being eight.

As far as the observations go, the sex chromosomes divide prereductionally in all the species. In the material there are a few species which may show bivalent auto-orientation, but this is by no means certain at present. Certain other interesting peculiarities shown by the material cannot be mentioned in this brief article.

Institute of Genetics. The University. Helsinki, Finland.

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